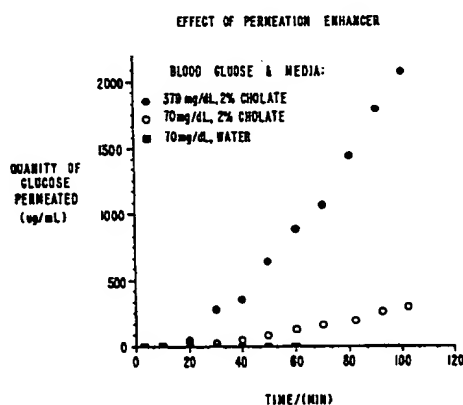




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>5</sup> :  A61B X	A2	(11) International Publication Number: WO 90/15568 (43) International Publication Date: 27 December 1990 (27.12.90)
(21) International Application Number: PCT/US90/02940 (22) International Filing Date: 24 May 1990 (24.05.90)  (30) Priority data: 360,360                      2 June 1989 (02.06.89)                      US  (71)(72) Applicants and Inventors: STANLEY, Theodore, H. [US/US]; 4800 Oak Terrace Drive, Salt Lake City, UT 84124 (US). EBERT, Charles, Dewey [US/US]; 558 North Perry's Hollow, Salt Lake City, UT 84103 (US). HIGUCHI, William, I. [US/US]; 3021 East Thackeray Place, Salt Lake City, UT 84108 (US). ZHANG, Jie [CN/US]; 3030 West 3725 South, Salt Lake City, UT 84119 (US).	(74) Agents: NYDEGGER, Rick, D. et al.; Workman, Nydegger & Jensen, 1000 Eagle Gate Tower, 60 East South Temple, Salt Lake City, UT 84111 (US).  (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>	

(54) Title: APPARATUS AND METHODS FOR NONINVASIVE BLOOD GLUCOSE MONITORING



## (57) Abstract

The present invention is directed to novel methods and apparatus for noninvasive blood glucose monitoring. Blood glucose is monitored noninvasively by correlation with amount of glucose which permeates an epithelial membrane (14), such as skin or a mucosal membrane, into a glucose receiving medium (12) over a specified time period. The glucose receiving (12) medium preferably includes a glucose permeation enhancer capable of increasing the glucose permeability across the epithelial membrane (14). The glucose receiving medium (12) is positioned against the epithelial membrane (14) so that the permeation enhancer alters the permeability of the membrane (14). After sufficient time delay, the glucose receiving medium (12) is removed and analyzed for the presence of glucose using conventional analytical techniques. The apparatus within the scope of the present invention includes means for supporting the glucose receiving medium. Such means for supporting the glucose receiving medium may include a housing (18) defining a receiving chamber therein which holds the glucose receiving medium (12) and an opening to the receiving chamber. The means for supporting the glucose receiving medium may also include a hydrogel. The apparatus also preferably includes means (20) for temporarily positioning the glucose receiving medium against the epithelial membrane.

### DESIGNATIONS OF "DE"

Until further notice, any designation of "DE" in any international application whose international filing date is prior to October 3, 1990, shall have effect in the territory of the Federal Republic of Germany with the exception of the territory of the former German Democratic Republic.

#### *FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MC	Monaco
AU	Australia	FI	Finland	MG	Madagascar
BB	Barbados	FR	France	ML	Mali
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Faso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GR	Greece	NL	Netherlands
BJ	Benin	HU	Hungary	NO	Norway
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	SD	Sudan
CF	Central African Republic	KP	Democratic People's Republic of Korea	SE	Sweden
CG	Congo	KR	Republic of Korea	SN	Senegal
CH	Switzerland	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
DE	Germany, Federal Republic of	LU	Luxembourg	TC	Togo
DK	Denmark			US	United States of America

1

**APPARATUS AND METHODS FOR NONINVASIVE  
BLOOD GLUCOSE MONITORING**

**BACKGROUND**

5

1. **The Field of the Invention**

The present invention is directed to methods and apparatus for monitoring blood constituents. More particularly, blood constituents such as glucose, are monitored noninvasively through diffusion across epithelial membranes.

2. **Technology Review**

The blood is routinely tested for various blood constituents in countless medical procedures. This typically involves drawing an actual blood sample from the patient, followed by blood analysis. Most people can tolerate giving an occasional blood sample, but continually drawing blood for analysis creates additional safety risks for the patient. For those persons suffering from diabetes mellitus (hereinafter referred to as "diabetes"), blood is often drawn many times a day.

Diabetes is a major health problem directly affecting over ten million people in the United States. The prevalence of the disease is increasing rapidly. Most people suffering from diabetes face the probability of major complications and shortened life spans. Diabetes is currently the seventh leading cause of death in the United States, and has been attributed to 35,000 to 50,000 deaths and costs of more than 20 billion dollars per year.

Diabetes is a disorder of carbohydrate metabolism characterized by elevated blood sugar (hyperglycemia), sugar in the urine (glycosuria), excessive urine production (polyuria), excessive thirst (polydipsia), and increase in

35

1 food intake (polyphagia). Diabetes is a chronic, incurable  
disease, but symptoms can be ameliorated and life prolonged  
by proper therapy. Diabetes results from the inadequate  
production or utilization of insulin.

5 Insulin is a hormone secreted by the pancreas which is  
essential for the proper metabolism of blood sugar  
(glucose) and for the maintenance of the proper blood  
glucose level. Severe insulin deficiency, or less severe  
insulin deficiency coupled with other conditions, can cause  
10 ketoacidosis which may lead to coma and life-threatening  
crisis.

Chronic diabetes is associated with vascular and  
neurologic degeneration, and persons with diabetes are at  
increased risk of heart disease, blindness, renal failure,  
15 and inadequate circulation and sensation in peripheral  
tissues. Women with diabetes also have increased risk of  
stillbirths and congenital malformations in their children.

These direct consequences of diabetes make it a  
disease that is costly and difficult to manage. However,  
20 the complications of diabetes are caused primarily by  
elevated blood glucose levels, and these complications can  
be avoided in most cases by monitoring and control of blood  
glucose levels and close medical supervision with  
appropriate intervention. Thus, blood sugar determinations  
25 may need to be made at frequent intervals in order to know  
if, when, and how much insulin is needed to control blood  
glucose.

Attempts have been made to monitor blood glucose  
noninvasively. As used in this specification, the term  
30 "noninvasive blood glucose monitoring" means determining  
blood glucose concentration without actually drawing the  
patient's blood. For example, efforts to monitor blood  
glucose based upon glucose concentration in a patient's  
saliva or breath have failed. The reason for these

1 failures is that there is no correlation between glucose in  
saliva or breath and the actual blood glucose levels. In  
fact, glucose does not naturally cross body membranes such  
as the buccal mucosa or membranes of the skin. Because  
5 most body membranes are naturally impermeable to glucose,  
the presence of glucose has historically been used to test  
whether tissue is intact in transdermal experiments.

In view of the foregoing, it will be appreciated that  
the development of apparatus and methods for noninvasive  
10 blood glucose monitoring would be a significant advancement  
in the art.

It would be another significant advancement in the art  
to provide apparatus and methods for noninvasive blood  
glucose monitoring which provide accurate and reproducible  
15 correlation with actual blood glucose levels.

Additionally, it would be a significant advancement in  
the art to provide apparatus and methods for noninvasive  
blood glucose monitoring which provide rapid results in  
sufficient time to administer appropriate medication.

20 Such apparatus and methods for noninvasive blood  
glucose monitoring are disclosed and claimed herein.

#### BRIEF SUMMARY OF THE INVENTION

The present invention is directed to novel methods and  
25 apparatus for noninvasive blood glucose monitoring.  
According to the present invention, blood glucose is  
monitored noninvasively by causing glucose to diffuse  
across epithelial membranes and then capturing and  
measuring that glucose for correlation to determine the  
30 blood glucose level. As used in this specification, the  
term epithelium or epithelial membrane refers to a variety  
of different dermal and mucosal surfaces.

In one preferred embodiment within the scope of the  
present invention, glucose diffuses across the buccal

1 mucosal membrane into a glucose receiving medium. The  
glucose receiving medium includes a permeation enhancer  
capable of increasing the glucose permeability across the  
mucosal membrane. The glucose receiving medium is  
5 positioned against the mucosal membrane so that the  
permeation enhancer alters the permeability of the mucosal  
membrane which it contacts. After sufficient time delay,  
the glucose receiving medium is removed and analyzed for  
glucose concentration using conventional analytical  
10 techniques.

In another preferred embodiment within the scope of  
the present invention, glucose diffuses across the skin  
into a glucose receiving medium. As described above, the  
glucose receiving medium includes a permeation enhancer  
15 capable of increasing the glucose permeability across skin.  
The glucose receiving medium is positioned against the skin  
so that the permeation enhancer alters the permeability of  
the skin which it contacts. After a predetermined time,  
the glucose receiving medium is removed and analyzed for  
20 glucose concentration. The glucose receiving medium is  
capable of releasing a permeation enhancer and receiving  
glucose.

The driving force behind glucose diffusion from blood  
into the glucose receiving medium is the glucose  
25 concentration gradient. The glucose receiving medium  
preferably has a relatively low glucose concentration for  
the entire duration of the measurement, while the  
interstitial fluid, which is in equilibrium with the  
capillary blood vessels perfusing the buccal mucosa and  
30 dermis, has a substantially higher glucose concentration.  
By enhancing the glucose permeability of the mucosal  
membrane or dermis, a measurable amount of glucose diffuses  
into the glucose receiving medium.

1       The apparatus within the scope of the present  
invention includes means for supporting the glucose  
receiving medium. Such means for supporting the glucose  
receiving medium may include a housing which holds and  
5 contains the glucose receiving medium. The means for  
supporting the glucose receiving medium may also include a  
hydrogel.

      The apparatus also includes means for temporarily  
positioning the glucose receiving medium against the  
10 mucosal membrane or skin. For example, if the apparatus  
includes a housing for supporting the glucose receiving  
medium, then an adhesive composition is preferably used to  
temporarily position the glucose receiving medium against  
the mucosal membrane. If the apparatus includes a hydrogel  
15 for supporting the glucose receiving medium, then the  
hydrogel itself may adhere directly to the mucosal membrane  
or skin.

      In addition, other means for temporarily positioning  
the glucose receiving medium against the mucosal membrane  
20 may include a lollipop-like configuration wherein a stick  
or holder aids in properly positioning the device against  
the mucosal membrane. Alternatively, a bandage or wrap  
configuration may be used to temporarily position the  
glucose receiving medium against the skin.

25       An important feature within the scope of the present  
invention is the use of a rate limiting membrane or medium.  
It will be appreciated that the overall rate that glucose  
diffuses through the epithelial membrane into the glucose  
receiving medium depends upon the individual glucose  
30 permeabilities of the membranes or media that the glucose  
must pass through to enter the glucose receiving medium.  
The overall glucose diffusion rate is determined by the net  
resistance of all diffusional components, the net diffusion  
being dominated by the single diffusion component with the

1 lowest glucose permeability. Thus, if a rate limiting  
membrane or medium having a precise and reproducible  
permeability is used, the overall glucose diffusion rate  
may be maintained relatively constant despite variations in  
5 epithelial membrane permeability from person to person,  
time to time, and even position to position.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a cross-sectional schematic view of the  
10 noninvasive glucose monitoring process of the present  
invention.

Figure 2 is a cross-sectional schematic view of the  
noninvasive glucose monitoring process of the present  
invention using a rate regulating membrane.

15 Figure 3 is a cross-sectional view of one possible  
apparatus within the scope of the present invention.

Figure 4 is a graph of glucose permeated ( $\mu\text{g}$ ) verses  
time for the results of Example 1.

Figure 5 is a graph of glucose permeated ( $\mu\text{g}/\text{cm}^2$ )  
20 verses time for the results of Examples 2 and 3.

Figure 6 is a graph of glucose flux ( $\mu\text{g}/\text{min}/\text{cm}^2$ ) verses  
blood glucose level ( $\text{mg}/\text{dl}$ ).

Figure 7 is a graph of glucose permeated ( $\mu\text{g}$ ) verses  
time for the results of Example 4.

25 Figure 8 is a graph of glucose permeated ( $\mu\text{g}$ ) verses  
time for the results of Example 5.

Figure 9 is a graph of glucose permeated ( $\mu\text{g}$ ) verses  
time for the results of Example 6.

Figure 10 is a graph of glucose permeated ( $\mu\text{g}/\text{cm}^2$ )  
30 verses blood glucose ( $\text{mg}/\text{dl}$ ) for the results of Examples 18  
and 19.

Figure 11 is a graph of glucose permeated ( $\mu\text{g}/\text{cm}^2$ )  
verses blood glucose ( $\text{mg}/\text{dl}$ ) for the results of Example 20.



1        Figure 12 is a graph of glucose permeated ( $\mu\text{g}/\text{cm}^2$ )  
verses blood glucose ( $\text{mg}/\text{dl}$ ) for the results of Example 21.

Figure 13 is a graph of glucose permeated ( $\mu\text{g}/\text{cm}^2$ )  
verses time for the results of Example 22.

5        Figure 14 is a graph of glucose permeated ( $\mu\text{g}/\text{cm}^2$ )  
verses time for the results of Examples 23 and 24.

Figure 15 is a graph of glucose permeated ( $\mu\text{g}/\text{cm}^2$ )  
verses time for the results of Example 26.

Figure 16 is a graph of glucose solubility ( $\text{mg}/\text{ml}$ )  
10 verses water content for the results of Example 27.

Figure 17 is schematic view of a diffusion cell used  
to perform in vitro skin flux experiments.

Figure 18 is a graph of glucose permeated ( $\mu\text{g}/\text{cm}^2$ )  
verses time (hours) for the results of Example 28.

15        Figure 19 is a graph of glucose permeated ( $\mu\text{g}/\text{cm}^2$ )  
verses time (hours) for the results of Example 29.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is directed to novel methods and  
20 apparatus for noninvasive blood glucose monitoring. Such  
noninvasive techniques avoid the inconvenience and risks  
associated with traditional invasive glucose monitoring  
techniques because blood does not need to be drawn from a  
patient.

25        According to the present invention, blood glucose is  
monitored noninvasively by correlation with amount of  
glucose which permeates an epithelial membrane into a  
glucose receiving medium over a specified time period. As  
used in this specification, the term epithelium or  
30 epithelial membrane refers to both dermal and mucosal  
surfaces of the body. Epithelial membranes are generally  
impermeable to glucose; therefore, a glucose permeation  
enhancer capable of increasing the glucose permeability  
across the epithelial membranes is preferably included in

1 the glucose receiving medium in order to enable glucose to  
back-diffuse from the interstitial fluid into the glucose  
receiving medium. Alternatively, it may be useful to  
pretreat the epithelial membrane with a permeation enhancer  
5 before applying the glucose receiving medium.

#### I. Theoretical Considerations

According to the apparatus and methods within the  
scope of the present invention, glucose diffuses from the  
10 interstitial fluid, which is in equilibrium with capillary  
blood vessels perfusing the epithelial membrane, across the  
membrane into a glucose receiving medium. From a  
theoretical viewpoint, the interstitial fluid in  
equilibrium with the capillary vessels is considered a  
15 donor chamber, and the glucose receiving solution is  
considered a receiver chamber. The donor chamber and the  
receiver chamber are separated by the epithelial membrane.  
In the donor chamber, there is a finite concentration ( $C_0$ )  
of permeant corresponding to glucose. The permeant  
20 concentration in the receiver chamber is zero at an initial  
time  $t=0$ .

If the concentration of the permeant in the donor  
chamber is kept constant ( $C_0$ ) and the permeant concentration  
in the receiver chamber is much lower than  $C_0$  for the entire  
25 experiment, then the amount of the permeant in the receiver  
chamber as a function of time is approximated by:

$$Q(t) = A \cdot C_0 \left[ \frac{Dt}{h} - \frac{h}{6} - \frac{2h}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} e^{-n^2 \pi^2 Dt/h^2} \right]$$

30

the diffusion lag time  $t(\text{lag})$  is given by:

$$t(\text{lag}) = \frac{h^2}{6D}$$

35

1 and the flux of the permeant is given by:

$$\text{Flux} = \frac{1}{A} \cdot \frac{dQ(t)}{dt}$$

5 where "Q(t)" is the mass of the permeant in the receiver chamber at time "t", "D" is the diffusion coefficient of the permeant in the membrane, "h" is the thickness of the membrane, "t" is time, and "A" is the area of the membrane. See Flynn, et al., 63 Journal of Pharmaceutical Sciences 479 (1974).

10 From the foregoing equations both Q(t) and flux are proportional to C<sub>o</sub> at any time. Since both Q(t) and flux(t) are at all times proportional to the glucose concentration in the blood, C<sub>o</sub>, they can be used to monitor the blood glucose concentration. However, in practice, precise  
15 measurement of Q(t) and the flux can only be made after t(lag) since Q(t) before t(lag) is very low.

Reference is now made to Figure 1. The foregoing equations are valid in those situations where the permeability coefficient of glucose receiving medium 12 is  
20 substantially greater than that of epithelial membrane 14. In such cases, glucose receiving medium 12 acts as a glucose "sink." The quantity of glucose diffusing from interstitial fluid 16, through the epithelial membrane 14, and into glucose receiving medium 12, is determined by the  
25 most resistant layer along the diffusion pathway -- epithelial membrane 14.

On the other hand, if the permeability coefficient of glucose receiving medium 12 is substantially less than that of epithelial membrane 14, than the quantity of glucose  
30 entering the glucose receiving medium is limited by glucose receiving medium 12.

Referring now to Figure 2, one or more additional membranes 26 may be placed between the epithelial membrane

1 and the glucose receiving medium to achieve a rate  
regulating function. In this case, it is preferred that  
the permeability coefficient of rate regulating membrane 26  
is significantly lower than that of epithelial membrane 14  
5 and that of glucose receiving medium 12. Thus, the  
quantity of glucose entering the glucose receiving medium  
is limited or regulated by the most resistant layer of the  
glucose diffusion pathway -- membrane 26.

From a theoretical viewpoint, glucose must diffuse  
10 through two membranes to enter the glucose receiving  
medium: the epithelial membrane and the rate regulating  
membrane. The effective permeability coefficient of the  
combined membranes is given by:

15 
$$P_T = P_M \cdot P_R / (P_M + P_R)$$

where " $P_T$ ", " $P_M$ ", and " $P_R$ " are the permeability coefficients  
of the combined membrane, the epithelial membrane, and the  
rate regulating membrane, respectively.  $P_R$  can be made  
20 precisely and reproducibly by modern techniques, while  $P_M$   
varies from person to person, time to time, and even  
position to position within the same type of epithelial  
membrane.

The above equation suggests that if  $P_R$  is significantly  
25 lower than  $P_M$ ,  $P_T$  will stay relatively stable despite  
variations in  $P_M$ . For example, if  $P_R = 1/3 P_M$ , a 30%  
variation in  $P_M$  will only cause about a 6% variation in  $P_T$ .

In a more sophisticated device within the scope of the  
present invention, a rate regulating medium or membrane is  
30 used. In the case of a rate regulating medium, the  
permeability coefficient of the glucose receiving medium is  
significantly lower than that of the epithelial membrane.  
In the case of the rate regulating membrane, one or several

1 membranes are placed between the epithelial membrane and  
the glucose receiving membrane.

In both of the above cases the quantity of glucose  
permeated into the glucose receiving medium is given by

5

$$Q(t) = C_0 \cdot f(A, D_M, D_R, h_M, h_R, t)$$

where "C<sub>0</sub>" is the concentration of the glucose in the  
interstitial fluid or capillary blood vessels; "A" is the  
10 area of contact; "D<sub>M</sub>" and "D<sub>R</sub>" are diffusion coefficients of  
glucose in the epithelial membrane and in the rate  
regulating membrane, respectively; "h<sub>M</sub>" and "h<sub>R</sub>" are the  
thicknesses of the epithelial membrane and the rate  
regulating membrane, respectively; "t" is the time passed  
15 from the beginning of contact; and "f" is a complicated  
function of the above variables. This equation is valid at  
all conditions, even if the "sink condition" is not  
maintained.

From the above equation, it is evident that the  
20 quantity of glucose permeated into the glucose receiving  
medium is proportional to the glucose concentration in the  
donor chamber (interstitial fluid and capillary blood  
vessels) at any time, provided that other variables in the  
above equation are kept relatively constant.

25 In a broad sense, the apparatus of the present  
invention is directed to a glucose receiving medium and to  
means for supporting the glucose receiving medium against  
an epithelial membrane. Permeation enhancers added to the  
glucose receiving medium alter the diffusion coefficient of  
30 glucose in the mucosal membrane thereby increasing Q(t) and  
flux and reducing t(lag). Without a permeation enhancer,  
the epithelial membrane is effectively impermeable to  
glucose. It has also been found that increasing the  
concentration or potency of the permeation enhancer

35

1 significantly reduces the diffusion lag time. In fact,  
dramatically increasing the permeation enhancer  
concentration renders the lag time substantially negligible  
and enables rapid detection of the blood glucose level  
5 noninvasively.

## II. Preferred Apparatus of the Present Invention

Reference is made to Figure 3 wherein one possible  
apparatus within the scope of the present invention is  
10 illustrated in a cross-sectional view. Noninvasive glucose  
monitoring device 10 includes glucose receiving medium 12.  
Glucose receiving medium 12 is positioned against  
epithelial membrane 14. Glucose receiving medium 12  
includes a permeation enhancer (not shown) for improving  
15 the glucose permeability of epithelial membrane 14.

Glucose is preferably soluble in the glucose receiving  
medium. Hence, water is one currently preferred glucose  
receiving medium. Other compositions which dissolve  
glucose may also be suitably used as glucose receiving  
20 media within the scope of the present invention.

Suitable glucose receiving media should not  
unfavorably react with glucose or the permeation enhancer.  
The glucose receiving medium preferably does not interfere  
with glucose concentration measurements. It should also be  
25 nontoxic to the epithelial membrane and chemically and  
physically stable (e.g., does not degrade and nonvolatile).

It is also within the scope of the present invention  
to provide a glucose receiving medium containing a  
complexing agent which selectively combines with glucose to  
30 form an insoluble product. If such a complexing agent is  
included in the glucose receiving medium, then the  
resulting glucose complex is preferably detectable using  
known analytical techniques. The resulting insoluble  
glucose product may facilitate quantifying the glucose

1 concentration. Lecithins and other sugar binding materials  
may be suitably used.

Typical permeation enhancers capable of improving the  
glucose permeability across the epithelial membrane include  
5 many natural bile salts such as sodium cholate, sodium  
glycocholate, sodium glycodeoxycholate, taurodeoxycholate,  
or sodium deoxycholate. Other permeation enhancers such as  
sodium lauryl sulfate, salts and other derivatives of  
saturated and unsaturated fatty acids, surfactants, bile  
10 salt analogs, derivatives of bile salts, or such synthetic  
permeation enhancers as described in United States Patent  
No. 4,746,508 may also be used.

It has been found that the effectiveness of some  
enhancers varies depending on the type of epithelium. For  
15 example, some enhancers known to improve skin glucose  
permeability are not as effective when used to enhance  
mucosal membrane permeability. The following enhancers are  
particularly effective when used with the some mucosal  
membranes (nasal, rectal, gastrointestinal), but less  
20 effective when used with buccal mucosal membranes: IGEPAL  
10.5 (octylphenoxy poly(ethoxyethanol) 10.5), EDTA  
(ethylene-diaminetetraacetic acid), sodium oleate, and  
sodium taurocholate (a bile salt).

In addition, the effectiveness of some enhancers may  
25 vary depending on the chemical compound to be permeated.  
The following enhancers effectively alter epithelial  
permeability with respect to certain drugs, but are not  
very effective at promoting glucose permeability: ethanol,  
low chain alcohols, and solvent-type enhancers used  
30 transdermally.

The following enhancers have been found to be  
particularly effective at enhancing the transmucosal  
glucose permeability: sodium cholate, sodium dodecyl  
sulfate, sodium deoxycholate, and taurodeoxycholate. The

1 following enhancers have been found to be particularly  
 effective at enhancing the dermal glucose permeability  
 individually or in combination: ethanol/water (with or  
 without cell envelope disordering compounds), dimethyl  
 5 sulfoxide (DMSO)/water, and isopropyl alcohol with methyl  
 laurate (a cell envelope disordering compound).

The enhancer concentration within the receiving medium  
 may be varied depending on the potency of the enhancer and  
 the desired epithelial membrane. Other criteria for  
 10 determining the enhancer concentration include the  
 sensitivity of the glucose detection methods, the desired  
 lag time. The upper limit for enhancer concentration is  
 set by toxic effect to or irritation limits of the  
 epithelial membrane. The solubility of the enhancer within  
 15 the receiving medium may also limit enhancer concentration.

The following is a list of typical enhancers and an  
 exemplary concentration range for each enhancer:

	<u>Enhancer</u>	<u>Operational Concentration</u>	<u>Preferred Range</u>
20	sodium cholate	0.1% - 50%	1%-16%
	sodium dodecyl sulfate	0.01% - 10%	0.1%-2%
	sodium deoxycholate	0.1% - 50%	1%-16%
25	taurodeoxycholate	0.1% - 50%	1%-16%
	sodium glycocholate	0.1% - 50%	1%-16%
	sodium taurocholate	0.1% - 50%	1%-16%
	IGEPAL 10.5	0.05% - 15%	0.5%-5%
30	EDTA	0.01% - 5%	0.1%-1%
	sodium oleate	0.1% - 10%	0.5%-5%
	DMSO	0.1% - 99%	5%-50%
35	Methyl Laurate in IPA*	0.1% - 20%	0.5%-5%



1	Glyceryl Monooleate in IPA*	0.1% - 60%	0.5%-5%
	Ethanol	5% - 100%	10%-50%

5       \* IPA = isopropyl alcohol

      The driving force behind glucose diffusion into glucose receiving medium 12 is the glucose concentration gradient between the interstitial fluid 16 and receiving medium 12. The resistance to the permeation is determined  
10 by the permeability of epithelial membrane 14, the permeability of glucose receiving medium 12, or some other rate regulating membrane or media.

      The glucose receiving medium preferably has a relatively low glucose concentration for the entire  
15 duration of measurement with respect to that in the interstitial fluid and capillary blood vessels, yet at the end of the measurement, the glucose concentration in the receiving medium is high enough to be measured precisely. By enhancing the glucose permeability of the epithelial  
20 membrane, a measurable amount of glucose diffuses into the glucose receiving medium.

      The actual amount of glucose which diffuses into the glucose receiving medium depends upon many factors such as the type of epithelial membrane, the enhancer used, the  
25 enhancer concentration, the contact exposure time, the type of glucose receiving medium, the surface area in contact with the glucose receiving medium, intimacy of contact between the glucose receiving medium and the membrane, and the solubility of glucose in the glucose receiving medium.  
30 It is within the skill in the art to modify the foregoing factors in order to cause the desired amount of glucose to diffuse into the glucose receiving medium.

1       The amount of glucose which must diffuse into the  
glucose receiving medium in order to be accurately measured  
depends upon the sensitivity of the analytical glucose  
detection methods used. Currently, techniques which detect  
5 glucose having a concentration as low as 0.5  $\mu\text{g/ml}$  provide  
suitable results for the diagnostic methods within the  
scope of the present invention. Other criteria useful in  
selecting a suitable glucose detection method include the  
glucose specificity and convenience of the method.

10       A housing 18 encloses the glucose receiving medium and  
protects the glucose receiving medium from potential  
glucose contamination sources such as saliva. Hence, one  
important function of housing 18 is to isolate the glucose  
receiving medium from glucose contamination sources.  
15 Another important function of housing 18 is to provide  
support for the glucose receiving medium. Housing 18 is  
preferably constructed of a material which is nontoxic,  
chemically stable, nonreactive with glucose and the  
permeation enhancers used, and inexpensive. Suitable  
20 materials include: polyethylene, polyolefins, polyamides,  
polycarbonates, vinyl polymers, and other similar materials  
known in the art.

Housing 18 may take many different shapes; however,  
the housing should define a chamber for holding a quantity  
25 of glucose receiving medium and provide an opening such  
that the glucose receiving medium may be placed directly  
against the epithelial membrane. Housing 18 may also  
include flanges 20 located about the periphery of the  
housing for receiving an adhesive so that the housing may  
30 be maintained in position against epithelial membrane 14.  
Housing 18 may also include an access port (not shown)  
through which glucose receiving medium may be introduced  
into the housing or through which the glucose receiving  
medium may be directly tested for glucose or removed for

1 external testing while the housing is maintained in position against the epithelial membrane.

When housing 18 is to be used for measuring glucose diffusion across a mucosal membrane, such as those in the mouth, a handle 22 may optionally be attached to housing 18 to facilitate placement and removal of the apparatus. Handle 22 is particularly desirable to provide user-control of placement and removal and to maintain housing 18 in contact with the mucosal tissues.

10 It should be noted that the apparatus within the scope of the present invention does not require a housing as illustrated in Figure 3. Other means for supporting and positioning glucose receiving medium 12 against epithelial membrane 14 may be used. For example, it has been found 15 that hydrogels not only provide suitable support for the glucose receiving medium, but also adhere to epithelial membranes, particularly mucosal membranes.

When a hydrogel is used to support the glucose receiving medium, the bioadhesive hydrogel itself may be 20 used to temporarily position the glucose receiving medium against the epithelial or mucosal membrane. In the context of a hydrogel, the glucose receiving medium corresponds to the aqueous portion of the hydrogel, whereas the cellulose frame or other material forming the hydrogel provides the 25 necessary support of the glucose receiving medium. Hence, the glucose receiving medium within the scope of the present invention may be supported in a hydrogel.

Importantly, many hydrogels are inherently sticky. Such bioadhesive hydrogels adhere directly to mucosal 30 tissues. Cellulose, including hydroxypropylcellulose and other cellulose derivatives known in the art, carbopol, gelatin, and other known substance which produce hydrogels may be used within the scope of the present invention.

1        Other support substances which perform substantially  
the same function as hydrogels may also be used. For  
example, creams, emulsions, suspensions, and other solid  
and semisolid media may also provide suitable support for  
5 a glucose receiving medium. However, glucose may not be as  
soluble in a nonaqueous glucose receiving medium  
incorporated into such media. A sponge-like embodiment may  
also provide suitable support for a glucose receiving  
medium.

10       Whether a hydrogel or other substance is used to  
support a glucose receiving medium, it is important that  
the glucose receiving medium be safely supported and  
maintained in contact with the epithelial tissues for  
sufficient time to effect glucose diffusion across the  
15 epithelial membrane.

In some embodiments within the scope of the present  
invention, the hydrogel or other support substance may be  
preferably covered or sealed from potential contaminants  
such as saliva. A nonpermeable membrane, such as a thin  
20 plastic layer, would protect the hydrogel from potential  
contamination. A housing, as described above, would also  
suitably protect the hydrogel. In addition, a housing (and  
optionally a handle) would facilitate positioning and  
removal of the hydrogel.

25       Depending on the thickness and total surface area of  
the hydrogel or other support substances, the edges may not  
need to be covered or sealed. In this regard, lateral  
edges 24, illustrated in Figures 1 and 2, may not be  
necessary. Of course, if there is a significant risk of  
30 glucose contamination from saliva or foods, then it would  
be important to include lateral edges 24.

It will be appreciated that there are many other  
possible embodiments within the scope of the present  
invention which perform substantially the same function as

1 the embodiment illustrated in Figure 3. For example,  
clamps, buccal tapes, matrix patch type designs, and  
designs similar to the transdermal or transmucosal patches  
described in the patent literature may be used within the  
5 scope of the present invention.

### III. Use of the Present Invention

In use, the glucose receiving medium is preferably  
positioned directly against the epithelial membrane so that  
10 the permeation enhancer contacts the epithelial membrane  
and increases the glucose permeability of the membrane.  
Various epithelial membranes, including mucosal and dermal  
surfaces of the body, may be utilized within the scope of  
the present invention.

15 Some epithelial membrane surfaces are more preferable  
than others. For example, post auricular skin is  
preferable over the palm of the hand. The selection of a  
suitable epithelial membrane depends upon a number  
criteria, such as glucose permeability for a given quantity  
20 of enhancer, degree of irritation caused by the enhancer,  
lag time, convenience (e.g., buccal membrane is more  
accessible than nasal and rectal membranes), and the degree  
of vascularization.

After sufficient time delay, the glucose receiving  
25 medium is removed and analyzed for the presence of glucose  
using conventional analytical techniques. Variables  
affecting sufficient exposure time include: glucose  
detecting sensitivity, permeability, lag time, enhancer  
concentration, glucose receiving medium surface contact  
30 area to volume ratio, and temperature.

There are many different techniques known in the art  
for determining glucose concentration. For those  
experiments using transmucosal glucose detection methods,  
the glucose which diffused across the mucosal membrane was

1 measured using a standard Glucose Diagnostic Kit Solution  
obtained from Sigma Chemical Co. (cat. #315-100). The  
procedure was modified slightly by using a glucose sample  
solution instead of blood. Glucose concentration as low as  
5 0.5  $\mu\text{g/ml}$  was accurately detected using this technique. In  
the transdermal glucose detection experiments, the glucose  
which diffused across the skin was measured using reverse-  
phase high pressure liquid chromatography ("HPLC").  
Glucose concentration as low as 60  $\mu\text{g/ml}$  was accurately  
10 detected using HPLC.

Because the glucose concentration within the glucose  
receiving medium is proportional to the patient's actual  
blood glucose level, once the glucose within the glucose  
receiving medium is determined the actual blood glucose  
15 level may be quickly calculated.

For user convenience, it may be desirable to  
incorporate a color indicator into the glucose receiving  
medium. In this way, the glucose concentration may be  
quickly determined by comparing any color change (not  
20 necessarily in the visible light spectrum) of the glucose  
receiving medium against a standard color chart. Examples  
of commercially available indicators include a combination  
of glucose oxidase, 4-aminoantipyrine, and p-  
hydroxybenzenesulfonate.

25

#### IV. Examples

The use of the methods for noninvasively monitoring  
blood glucose concentration within the scope of the present  
invention will be further clarified by a consideration of  
30 the following examples, which are intended to be purely  
exemplary of the use of the invention and should not be  
viewed as a limitation on any claimed embodiment.

35

1 Example 1

In this example, blood glucose was monitored noninvasively in a laboratory dog. A 0.5 mm - 1.0 mm thick layer of silicone grease was spread on the base of a diffusion cell to provide the adhesiveness and prevent leakage of the glucose receiving solution in the cell. The diffusion cell had an open bottom designed to be placed on the dog's buccal mucosa and an open top through which glucose receiving solution was added and removed. The area of the cell's open bottom was 1.89 cm<sup>2</sup>. The diffusion cell was placed on the dog's buccal mucosa.

At time  $t=0$ , 2 ml of a glucose receiving solution were pipetted into the cell through the cell's open top. The glucose receiving solution was a 2% by weight solution of sodium cholate in deionized (DI) water. The glucose receiving solution was in direct contact with the buccal mucosa through the open bottom end of the diffusion cell.

One (1) ml of solution was withdrawn from the cell after 3 minutes. One (1) ml of fresh glucose receiving solution was pipetted into the cell immediately after sampling to maintain the volume of the solution in the cell at 2 ml. Sampling was repeated at time  $t=10$  minutes and at 10 minute intervals thereafter up to 120 minutes. The dilution due to the replacement with the fresh glucose receiving solution was corrected for in the calculation of total amount of glucose permeated.

Each 1 ml sample from the diffusion cell was placed in a glass vial containing 2 ml of a standard Glucose Diagnostic Kit Solution (Sigma Chemical Co., cat. #315-100). The resultant 3 ml solution was incubated at room temperature for exactly 18 minutes. After incubation, the 3 ml solution was placed in a 1 cm path length cuvette cell. The absorbance of the mixture at 505 nanometers was measured by a colorimeter (Milton Roy, Spectronic 21).

1 The relationship between the glucose concentration in  
the sample and the absorbance is given by the following  
equation:

$$C (\mu\text{g/ml}) = A + B \times \text{Absorbance}$$

5 where A and B are constants determined by testing standard  
glucose concentration solutions. The glucose concentration  
in each sample, and from which the total amount of glucose  
permeated across the buccal mucosa at a given time, can  
thus be obtained. The experimental results of Example 1  
10 are given in Table I and shown graphically in Figure 4.

Table I

<u>n</u>	<u>t/min</u>	<u>absorbance</u>	<u>C in sample</u> <u>(<math>\mu\text{g/mL}</math>)</u>	<u>Glucose</u> <u>permeated (<math>\mu\text{g}</math>)</u>
1	3.00	0.103	0.17	0.34
15 2	10.00	0.112	1.38	2.93
3	20.00	0.142	5.15	11.85
4	30.00	0.278	23.71	54.12
5	40.00	0.430	44.16	118.73
6	50.00	0.418	42.55	159.67
7	60.00	0.513	55.33	227.78
8	70.00	0.558	61.38	295.21
9	80.00	0.600	67.03	367.89
20 10	90.00	0.565	62.32	425.50
11	100.00	0.544	59.50	482.18
12	110.00	0.570	63.00	548.68
13	120.00	0.670	76.45	638.58

The total amount of glucose permeated is given by the  
25 following formula:

$$G_n = C_n \cdot V_{\text{receiver}} + \left( \sum_{i=1}^{n-1} C_i \right) \cdot V_{\text{sample}}$$

30 where " $G_n$ " is total amount of glucose permeated ( $\mu\text{g}$ ) through  
sample number n and " $C_n$ " is the glucose concentration  
( $\mu\text{g/ml}$ ) in sample n ( $C_n = 0$  when  $n = 0$ ), " $V_{\text{receiver}}$ " is the



1 volume of receiver chamber fluid (2 ml), and " $V_{\text{sample}}$ " is the  
volume of sample withdrawn from the cell (1 ml).

The actual blood glucose level was monitored by taking  
blood samples from the femoral artery about every 10 to 15  
5 minutes for the whole duration of the procedure. The  
glucose concentration in the blood samples were determined  
by the combination of Glucostix (Ames 2628C) and Glucometer  
(type II, model 5625, Ames Division, Miles Labs, Inc., P.O.  
Box 70, Elkhart, IN 46515). The standard procedure as  
10 described in the user's manual of the Glucometer was  
followed.

#### Example 2

Blood glucose was monitored noninvasively in a  
laboratory dog according to the procedure of Example 1,  
15 except that the blood glucose was artificially elevated.  
The elevation of the dog's blood glucose concentration was  
achieved by intravenous infusion of a glucose solution.  
The infusion rate was adjusted for the individual dog to  
achieve the desired blood glucose level.

20 With the particular dog used in this example, 6  
grams/hour glucose was needed to increase the blood glucose  
level from about 97 mg/dl to about 211 mg/dl. It required  
about 2 hours to establish an elevated steady state blood  
glucose level. The experimental results of Example 2,  
25 along with that of Example 3, are shown graphically in  
Figure 5.

The results of this example demonstrate that the  
glucose back diffusion flux, as well as the amount of  
glucose permeated, do reflect the actual blood glucose  
30 level change.

#### Example 3

Blood glucose was monitored noninvasively in a  
laboratory dog according to the procedure of Example 1,  
except that the blood glucose was artificially suppressed.

1 The same laboratory dog used in Example 2 was used in this  
Example. The suppression of the dog's blood glucose  
concentration was achieved by intravenous infusion of an  
insulin solution. The infusion rate was adjusted for the  
5 individual dog to achieve the desired blood glucose level.

With the particular dog used in this example, 0.9  
U/hour insulin was needed to reduce the blood glucose level  
from about 97 mg/dl to about 34 mg/dl. It required about  
2 hours to establish a suppressed steady state blood  
10 glucose level. Additional adjustment of the insulin  
infusion rate was necessary to maintain the glucose level  
relatively constant. The experimental results of Example  
3, along with that of Example 2, are shown graphically in  
Figure 5.

15 The results of this example demonstrate that the  
glucose back diffusion flux, as well as the amount of  
glucose permeated, do reflect the actual blood glucose  
level change.

The glucose back diffusion flux of Examples 2 and 3  
20 may be determined from the slope of the data plotted in  
Figure 5. The glucose flux ( $\mu\text{g}/\text{min}/\text{cm}^2$ ) was calculated and  
plotted in Figure 6. The results of Figure 6 demonstrate  
that glucose flux is proportional to the blood glucose  
level. Thus, by determining glucose flux it is possible to  
25 determine actual blood glucose levels.

#### Example 4

Blood glucose was monitored noninvasively in a  
laboratory dog according to the procedure of Examples 1 and  
2, except that a glucose receiving solution without a  
30 permeation enhancer was tested in addition to the glucose  
receiving solutions having 2% sodium cholate. The  
experimental results of Example 4 are shown graphically in  
Figure 7. These results indicate that no glucose permeated  
the buccal mucosa without a permeation enhancer.

### Example 5

Blood glucose was monitored noninvasively in a laboratory dog according to the procedure of Example 1, except that the glucose receiving solution was a 2% by weight solution of sodium cholate in 30% ethanol and 70% deionized (DI) water.

The experimental results of Example 5 compared with results using the procedure of Example 1 are shown graphically in Figure 8. These results indicate that the amount of glucose permeated when 30% ethanol is present in the glucose receiving solution is substantially lower than when no ethanol is present. It is currently believed that this result is due to the fact that the solubility of glucose in water is higher than the solubility in ethanol. Consequently, the tendency of glucose to permeate from plasma into the 30% ethanol solution is lower than into a deionized water solution.

### Example 6

Blood glucose was monitored noninvasively in a laboratory dog according to the procedure of Examples 1 and 3, except that the normal blood glucose level was about 100.4 mg/dl and that the suppressed blood glucose level was maintained about 32.8 mg/dl. The experimental results of Example 6 comparing the amount of glucose permeated ( $\mu\text{g}$ ) over time when the blood glucose is suppressed and when the blood glucose is normal are shown graphically in Figure 9. This example confirms that the blood glucose level changes may be monitored by the methods of the present invention.

### Examples 7-17

30 In this example, blood glucose was monitored noninvasively in a laboratory dog according to the procedure of Example 1, except that a variety of different permeation enhancers were examined to determine their respective mucosal glucose permeability enhancement

1 ability. The permeation enhancers tested in this example  
 were sodium cholate, sodium dodecyl sulfate, sodium  
 deoxycholate, taurodeoxycholate, sodium glycocholate,  
 IGEPAL 10.5, EDTA, sodium oleate, and sodium taurocholate.

5 A glucose receiving medium was prepared for each  
 respective permeation enhancer wherein the permeation  
 enhancer concentration was 2% by weight in deionized water,  
 except for EDTA which was tested at a concentration of 0.2%  
 and 1%. Sodium oleate was also tested at an 8%  
 10 concentration. Table II summarizes the experimental  
 results. Those enhancers which were classified "good"  
 resulted in diffusion of readily detectable quantities of  
 glucose across the mucosal membrane. Those enhancers which  
 were classified "poor" enabled barely detectable quantities  
 15 of glucose to permeate the mucosal membrane. Enhancers  
 classified "bad" did not enable detectable quantities of  
 glucose to permeate the mucosal membrane.

Table II

	<u>Example</u>	<u>Enhancer</u>	<u>Concentration</u>	<u>Results</u>
20	7	sodium cholate	2%	good
	8	sodium dodecyl sulfate	2%	good
	9	sodium deoxycholate	2%	good
	10	taurodeoxycholate	2%	good
	11	sodium glycocholate	2%	poor
25	12	sodium taurocholate	2%	poor
	13	IGEPAL 10.5	2%	bad
	14	EDTA	0.2%	bad
	15	EDTA	1%	bad
	16	sodium oleate	2%	bad
30	17	sodium oleate	8%	bad

1 Example 18

In this example, blood glucose was monitored noninvasively in a laboratory dog using a hydrogel. The hydrogel was prepared by placing 2 g of hydroxypropylcellulose in a glass vial. The glass vial was positioned in a 55°C water bath until temperature equilibrium was reached. Five (5) ml of 55°C deionized water was then introduced into the vial. The mixture was stirred thoroughly and a slurry was formed. Hydroxypropylcellulose is insoluble in water 45°C or warmer. Five (5) ml of room temperature deionized water containing 16% by weight sodium cholate was introduced into the vial. The mixture was stirred gently until a hydrogel was formed. The hydrogel was centrifuged for 45 minutes to remove any air bubbles. The resulting hydrogel had an 83% water content which contained 8% sodium cholate. The hydrogel was transparent and homogeneous.

Four slices of the hydrogel having a surface area of 1.04 cm<sup>2</sup> were placed on the buccal mucosa of a laboratory dog. Thirty (30) minutes later, two hydrogel slices were removed from the dog. Sixty (60) minutes later the remaining two hydrogel slices were removed. The actual blood glucose concentration for the dog was monitored during the experiment as described in Example 1. The average blood glucose concentration during the measurements was found to be about 100 mg/dl.

The hydrogel slices removed from the dog's buccal mucosa were weighed and placed in separate glass vials. Deionized water was added to the vials such that the combined weight of the hydrogel and the deionized water was 2.5 g. The vials were shaken until the hydrogel was swollen to at least double its original volume and a considerable part of the hydrogel was dissolved in the deionized water. The vials were shook for about 60

1 minutes. It was assumed that after 60 minutes of shaking, the glucose concentration in the gel and in the liquid are equal. This assumption has been confirmed experimentally.

One (1) ml of the solution was withdrawn from the vial  
5 and incubated with 2 ml glucose oxidase solution (Sigma, Glucose diagnostic Kit #315-100) for exactly 18 minutes. The absorbance of the mixture at 505 nanometers was measured by a colorimeter. The glucose concentration in the mixture, and from which the total amount of glucose in  
10 the hydrogel, was obtaining by using a calibration curve.

The calibration curve was prepared by placing five glucose solutions having glucose concentrations of C=0, 25, 50, 75, and 100  $\mu\text{g/ml}$  respectively, into five glass vials. 2 ml of the standard glucose solution were placed in the  
15 respective vial. Each glass vial contained 0.4 g of hydrogel described above and 0.1 g deionized water. The vials were shaken for 60 minutes. Assuming the glucose concentrations in the gel and the liquid reached equilibrium by the end of the shaking, the liquid part of  
20 each vial had glucose concentrations of C=0, 20, 40, 60, and 80  $\mu\text{g/ml}$ , respectively. The weight of the hydroxypropylcellulose (0.067 g) was ignored. One (1) ml of the liquid from each vial was incubated with 2 ml glucose oxidase solution for exactly 18 minutes, and the  
25 absorbance at 505 nanometers was measured. The absorbance verses glucose concentration relation was found to be:

$$C (\mu\text{g/ml}) = -14.82 + 130.45 \times \text{Absorbance}, R=1$$

30 Because this relation is very close to that in water, it is believed the glucose concentration equilibrium between the hydrogel part and the liquid part was reached after 60 minutes of shaking.

1       The total amount of glucose permeated into the  
hydrogel is shown in Figure 10 as a function of blood  
glucose concentration. These results indicate that the  
amount of glucose in the hydrogel remarkably reflects the  
5 change in the blood glucose concentration. The  
relationship between the glucose in the hydrogel and the  
blood glucose level were remarkably linear. Considering  
the uncertainty in the blood glucose level measurements by  
the Glucostix - Glucometer and the intrinsic blood glucose  
10 level fluctuation at both natural level and the manipulated  
(elevated or suppressed) level, it can be concluded that  
the deviation from the linearity is within the experimental  
uncertainty and the fluctuation of the blood glucose level  
in the dog.

15                               Example 19

Blood glucose was monitored noninvasively in a  
laboratory dog using a hydrogel according to the procedure  
of Example 18, except that the blood glucose level was  
about 140 mg/dl. The same laboratory dog used in Example  
20 18 was used in Example 19. The total amount of glucose  
permeated into the hydrogel is also shown in Figure 10 as  
a function of blood glucose concentration.

Example 20

Blood glucose was monitored noninvasively in a  
25 laboratory dog using a hydrogel according to the procedure  
of Example 18, except that the blood glucose level in the  
dog was elevated to about 245 mg/dl using the procedure  
described in Example 2. Blood glucose was also monitored  
in the same laboratory dog according to the procedure of  
30 Example 18, except that the normal blood glucose level was  
about 120 mg/dl. In both the normal and elevated blood  
glucose tests, the concentration of sodium cholate in the  
hydrogel was 4% by weight. In addition, the hydrogel was  
placed on the buccal mucosa only for a period of 60

1 minutes. The total amount of glucose permeated into the hydrogel, normalized to  $\mu\text{g}/\text{cm}^2$ , is shown in Figure 11 as a function of blood glucose concentration (mg/dl).

#### Example 21

5 Blood glucose was monitored noninvasively in a laboratory dog using a hydrogel according to the procedure of Example 18, except that the blood glucose level in the dog was elevated to about 210 mg/dl using the procedure described in Example 2. Blood glucose was also monitored  
10 noninvasively in the same laboratory dog using a hydrogel according to the procedure of Example 18, except that the normal blood glucose level was about 95 mg/dl. The total amount of glucose permeated into the hydrogel, normalized to  $\mu\text{g}/\text{cm}^2$ , for both the elevated and normal blood glucose  
15 levels is shown in Figure 12 as a function of blood glucose concentration (mg/dl).

#### Example 22

In this example, blood glucose was monitored noninvasively in a laboratory dog using a hydrogel. The  
20 hydrogel was prepared according to the procedure of Example 18, except that the concentration of sodium cholate in the hydrogel was 8%. Six hydrogel slices were placed on the buccal mucosa of a laboratory dog as described in Example 18. Two hydrogel slices were removed after 10, 20, and 30  
25 minutes, respectively, and analyzed according to the procedure of Example 18. The blood glucose level in the dog was about 136.8 mg/dl during the procedure. The total amount of glucose permeated into the hydrogel, normalized to  $\mu\text{g}/\text{cm}^2$ , verses time is shown in Figure 13. The lag time  
30 was determined to be 2.7 minutes.

#### Example 23

In this example, blood glucose was monitored noninvasively in a laboratory dog using a hydrogel. The hydrogel was prepared according to the procedure of Example



1 18, except that the concentration of sodium cholate in the  
hydrogel was 16%. In addition, the hydrogel slices were  
removed after 9 minutes and 16 minutes, rather than 30 and  
60 minutes. The blood glucose level in the dog was about  
5 109.3 mg/dl during the procedure. The total amount of  
glucose permeated into the hydrogel, normalized to  $\mu\text{g}/\text{cm}^2$ ,  
verses time is shown in Figure 14. The lag time was  
negligible.

#### Example 24

10 Blood glucose was monitored noninvasively in a  
laboratory dog using a hydrogel according to the procedure  
of Example 23, except that the blood glucose level was  
elevated according to the procedure described in Example 2.  
The same laboratory dog used in Example 23 was used in this  
15 Example. The blood glucose level in the dog was maintained  
about 189.5 mg/dl during the procedure. The total amount  
of glucose permeated into the hydrogel, normalized to  
 $\mu\text{g}/\text{cm}^2$ , verses time is also shown in Figure 14.

#### Example 25

20 Blood glucose was monitored noninvasively in a  
laboratory dog to determine the effect of surface area on  
glucose flux according to the procedure of Example 1,  
except that two differently sized diffusion cells were  
used. Each diffusion cell was placed on one side of the  
25 dog's mouth. Diffusion cell "A" had an open bottom area of  
 $1.887 \text{ cm}^2$ , while diffusion cell "B" had an area of  $0.866 \text{ cm}^2$ .  
The glucose flux for diffusion cell "A" was determined  
to be  $4.87 \mu\text{g}/\text{minute}/\text{cm}^2$ , while the glucose flux for  
diffusion cell "B" was determined to be  $4.80 \mu\text{g}/\text{minute}/\text{cm}^2$ .  
30 The results of Example 25 demonstrate that glucose flux is  
independent of surface area.

#### Example 26

Blood glucose was monitored noninvasively in a  
laboratory dog using a diffusion cell and a hydrogel

1 according to the procedures of Examples 1 and 18,  
respectively, except that the concentration of sodium  
cholate in both the glucose receiving solution and in the  
hydrogel was 8%.

5 It was found that the total amount of glucose  
permeated over time was lower by a factor of about 3 using  
the hydrogel than with the diffusion cell. This result  
suggests that glucose permeating into the hydrogel is a  
rate limiting step when compared to the permeation of  
10 glucose across the mucosal membrane. Therefore, the  
results of Example 26 suggest that use of a hydrogel may  
provide a substantially uniform glucose permeation rate  
which is substantially independent of individual variations  
in mucosal membrane permeability. The hydrogel in Example  
15 26 was acting as a rate regulating medium. The total  
amount of glucose permeated into the hydrogel, normalized  
to  $\mu\text{g}/\text{cm}^2$ , verses time is also shown in Figure 15.

#### Example 27

The solubility of glucose in various enhancer systems  
20 was determined by adding an excess amount of glucose to 5  
ml of the tested enhancer system. After 15 minutes of  
sonication, the suspension was equilibrated for 3 days with  
shaking in a 32°C water bath. The suspension was filtered  
through a Gelman ACRO LC13 filter (pore size, 0.2  $\mu\text{m}$ ), and  
25 the glucose concentration was determined by reverse-phase  
high pressure liquid chromatography ("HPLC"). The mobile  
phase was 80/16/2/2  $\text{CH}_3\text{CN}/\text{H}_2\text{O}/\text{NH}_4\text{OH}/\text{CHCl}_3$ . The injection  
volume was 100  $\mu\text{l}$ , and the flow rate was 2.0 ml/min at a  
column pressure of 1200 psi. A Whatman 11 cm x 3.9 mm  
30 Partisphere Polar Amino-Cyano column was used. The run  
time was from 5-7 minutes with a retention time of 3.4  
minutes.

Table III shows the solubility of glucose in ten  
different enhancer systems. In the  $\text{EtOH}/\text{H}_2\text{O}$  and  $\text{DMSO}/\text{H}_2\text{O}$

1 systems, glucose solubility is a linear function of H<sub>2</sub>O  
 content in the enhancer formulations. It ranges from 22  
 mg/ml to 275 mg/ml in the EtOH/H<sub>2</sub>O systems and ranges from  
 223 mg/ml to 235 mg/ml in the DMSO/H<sub>2</sub>O systems. Glucose  
 5 solubility data as a function of water content are  
 presented in Figure 16. In the 90/10 (40/60 ML/IPA)/H<sub>2</sub>O and  
 90/10 (40/60 GMO/IPA)/H<sub>2</sub>O systems, glucose solubilities are  
 9 and 7 mg/ml, respectively.

The enhancers with low glucose solubility will have a  
 10 lower diffusion driving force for glucose back flux,  
 resulting in lower quantities of glucose in the receiving  
 medium.

Table III  
 Solubility of Glucose in Various Enhancer  
 Systems at 32°C

15

Glucose Receiving Medium		Solubility (mg/ml)
20	1. 90/10 EtOH/H <sub>2</sub> O	22
	2. 80/20 EtOH/H <sub>2</sub> O	86
	3. 70/30 EtOH/H <sub>2</sub> O	152
	4. 60/40 EtOH/H <sub>2</sub> O	212
	5. 50/50 EtOH/H <sub>2</sub> O	275
25	6. 90/10 DMSO/H <sub>2</sub> O	223
	7. 80/20 DMSO/H <sub>2</sub> O	230
	8. 70/30 DMSO/H <sub>2</sub> O	235
30	9. 90/10 (40/60 ML*/IPA)/H <sub>2</sub> O	9
	10. 90/10 (40/60 GMO**/IPA)/H <sub>2</sub> O	7

\* ML = Methyl Laurate  
 \*\* GMO = Glyceryl Monooleate

35

1

Example 28

In order to examine the feasibility of the novel glucose back-diffusion concept toward the design of noninvasive transdermal glucose sensors, the in vitro back-diffusion kinetics of glucose has been measured across the human cadaver skin at 32°C.

The in vitro skin flux experiments were carried out using modified Franz diffusion cells such as those illustrated in Figure 17. Human cadaver skin was heat separated at 60°C for 1 minute and the epidermal layer was mounted between the donor and receptor compartments of the cells, the stratum corneum facing the receptor compartment. The diffusion cells were then placed in a heating/stirring module (Pierce Chemical-ReactiTherm®) for temperature control and stirring of the donor compartment. The surface temperature of skin was maintained at  $32 \pm 1^\circ\text{C}$ .

In this example, PBS saline containing a glucose concentration of 100 mg/ml, which is about 100 times higher than normal blood glucose level (~ 100 ml/dl), was introduced to the donor compartment which is the lower portion of the diffusion cell.

For the purpose of fluidizing the stratum corneum, which is the main barrier to the glucose permeation, and eliminating the lag time, a selected enhancer system from Table III was introduced to the receptor compartment for 24 hours. The receptor compartment was the upper portion of the diffusion cell illustrated in Figure 17. The enhancer system was then replaced by the same, but fresh, enhancer formulation and the samples were taken afterwards.

The back-diffusion of glucose into the enhancer systems were measured at 1, 2, and 24 hours after pretreatment with the enhancer.

35

1           Glucose was analyzed using reverse phase HPLC. The  
mobile phase was 80/16/2/2 CH<sub>3</sub>CN/H<sub>2</sub>O/NH<sub>4</sub>OH/CHCl<sub>3</sub>. The  
injection volume was 100 µl, and the flow rate was 2.0  
ml/min at a column pressure of 1200 psi. A Whatman 11 cm  
5 x 3.9 mm PartisSphere Polar Amino-Cyano column was used.  
The run time was from 5-7 minutes with a retention time of  
3.4 minutes.

This example was designed with the idea that a diabetic patient can apply a transdermal patch containing enhancer formulation to the test site on the body for a certain period of time before the blood glucose is read by another transdermal patch/sensor system.

Figure 18 shows the results obtained with four of the enhancer formulations identified in Table III (90/10 DMSO/H<sub>2</sub>O, 90/10 (40/60 ML/IPA)/H<sub>2</sub>O, 70/30 EtOH/H<sub>2</sub>O, and 8 M urea in H<sub>2</sub>O). The cumulative amount of glucose back-diffusing across skin was determined with each formulation up to 24 hours after the pretreatment stage in 1 to 3 skin donor samples (each composition evaluated in triplicate per donor skin).

A steady-state flux of only  $6.2 \mu\text{g}/\text{cm}^2/\text{hr}$  was obtained with 8 M urea/ $\text{H}_2\text{O}$  system. 70/30 EtOH/ $\text{H}_2\text{O}$ , the enhancer formulation used in Estraderm® patch, produced a steady-state flux of  $167.6 \mu\text{g}/\text{cm}^2/\text{hr}$  in the first 2 hours of study. However, the flux leveled off from 2 to 24 hours. This may be attributed to the ethanol depletion from the enhancer formulation. In contrast, 90/10 DMSO/ $\text{H}_2\text{O}$  and 90/10 (40/60 ML/IPA)/ $\text{H}_2\text{O}$  produced a steady-state flux rates of about  $89 \mu\text{g}/\text{cm}^2/\text{hr}$ , respectively, for up to 24 hours.

30 Example 29

Glucose skin flux determinations were made without pretreating the skin with an enhancer system. The skin was equilibrated with a glucose/PBS saline solution in the donor compartment for 4 hours. An enhancer system was then

1 introduced to the receptor compartments at time,  $t=0$ . The  
amount of glucose permeating across the skin was measured  
at 4, 8, 20, 24, and 28 hours. At those sampling times,  
the receptor solutions (3 ml) were removed and glucose  
5 concentrations were determined by HPLC. The receptor  
solutions were replaced with the same, but fresh, enhancer  
formulations immediately and the caps were closely  
tightened to prevent the evaporation of enhancer  
ingredients.

10 The cumulative amounts of glucose permeating across  
the skin at time  $= t$  ( $Q_t$ ,  $\mu\text{g}/\text{cm}^2$ ) were determined using the  
following equation:

15 
$$Q_t = \sum_{n=0}^t C_n \cdot V/S$$

Where " $C_n$ " is the glucose concentration ( $\mu\text{g}/\text{ml}$ ) determined  
by HPLC at time interval " $t$ ", " $V$ " is the receptor volume  
20 ( $\text{ml}$ ) and " $S$ " is the surface area ( $\text{cm}^2$ ). The steady-state  
flux, " $J_s$ " ( $\mu\text{g}/\text{cm}^2/\text{hr}$ ), was calculated from the linear slope  
of  $Q_t$  verses  $t$  curve. By extrapolating the steady-state  
flux curve to the x-axis, the lag time, " $t_l$ ", was  
determined.

25 The in vitro skin flux experiments in this example  
were conducted under more realistic conditions than in  
Example 28. The glucose concentration in PBS saline was  
reduced from 100  $\text{mg}/\text{ml}$  to 10  $\text{mg}/\text{ml}$  and 1  $\text{mg}/\text{ml}$  to mimic the  
hyperglycemic and normal blood level. The enhancer system  
30 was added to the receptor compartment and the samples were  
taken periodically without pretreating the skin. The  
purpose of this example was to determine the steady-state  
flux as well as the lag times required before reaching  
steady-state permeation.

1        Figure 19 shows the results obtained with the four  
enhancer systems used in Example 28 (90/10 DMSO/H<sub>2</sub>O, 90/10  
(40/60 ML/IPA)/H<sub>2</sub>O, 70/30 EtOH/H<sub>2</sub>O, and 8 M urea in H<sub>2</sub>O).

5        The glucose concentration was 1 mg/ml in the donor  
compartments and four enhancer systems were in the receptor  
compartments. In one study using 90/10 DMSO/H<sub>2</sub>O, a glucose  
concentration of 10 mg/ml was employed as the donor  
solution. In the case of 1 mg/ml glucose level, the  
steady-state flux rates ranged from 5.8 to 125.8  $\mu\text{g}/\text{cm}^2/\text{hr}$ ,  
10 with 70/30 EtOH/H<sub>2</sub>O producing the highest flux. However,  
70/30 EtOH/H<sub>2</sub>O also generated the longest lag time, 23.2  
hours. On the contrary, 90/10 DMSO/H<sub>2</sub>O produced time lags  
of approximately 1-2 hours. As anticipated, glucose back-  
flux was reduced by lowering the donor concentration from  
15 10 mg/ml to 1 mg/ml. However, this flux reduction was  
substantially less than 10-fold.

Although highly speculative, it is possible that  
glucose back-diffuses via a pore pathway which is viscosity  
dependent. As the glucose concentration decreases,  
20 viscosity also decreases in the donor solution. Such a  
viscosity effect would increase the diffusion coefficient  
at 1 mg/ml relative to that of a 10 mg/ml, thus accounting  
for the unexpectedly high flux at 1 mg/ml relative to 10  
mg/ml.

25        Table IV summarizes the results obtained from these in  
vitro skin flux studies. The average skin flux rate, lag  
time and skin permeability ( $P_s$ ) are tabulated. A  $P_s$  of  $1.06$   
 $\times 10^{-5}$  cm/sec was obtained from 90/10 DMSO/H<sub>2</sub>O under  
physiological conditions (i.e., glucose concentration in  
30 the donor side is 1 mg/ml). The  $P_s$  value of the enhancer  
treated skin was close to the epidermis/dermis permeability  
(i.e., a  $P_s$  of around 1 to  $5 \times 10^{-5}$  cm/sec) indicating that  
the barrier property of the stratum corneum was essentially  
abolished in this study.

1 Thus, by employing a rate-limiting membrane with a  
 permeability coefficient  $P_m < 1 \times 10^{-5}$  cm/sec, the glucose  
 flux may be controlled by the rate-limiting membrane. By  
 using a transdermal patch of about 10 cm<sup>2</sup> and a glucose  
 5 sensor with detection limit of  $\sim 1$   $\mu$ g/ml, a noninvasive  
 glucose sensor can be developed by this novel approach.

Table IV  
 Summary of In-Vitro Skin Flux Data

10	Enhancer Systems	Glucose Conc. (mg/ml)	Flux ( $\mu$ g/cm <sup>2</sup> /hr)	Lag Time (hrs)	$P_s \times 10^6$ (cm/sec)
	90/10 DMSO/H <sub>2</sub> O	1	38.2	2.5	10.61
	90/10 DMSO/H <sub>2</sub> O	10	101.3	1.0	2.81
15	90/10 DMSO/H <sub>2</sub> O	100	229.5	--	0.64
	90/10 */H <sub>2</sub> O	1	15.7	19.0	4.36
	90/10 */H <sub>2</sub> O	100	88.6	--	0.25
	70/30 EtOH/H <sub>2</sub> O	1	125.8	23.2	34.94
	70/30 EtOH/H <sub>2</sub> O	100	167.6	--	0.47
20	8 M urea/H <sub>2</sub> O	1	5.8	5	1.61
	8 M urea/H <sub>2</sub> O	100	6.2	--	0.02

\* = 40/60 methyl laurate/isopropyl alcohol

In summary, the present invention permits noninvasive  
 25 blood glucose monitoring which can be performed nearly as  
 rapidly as conventional monitoring techniques, but without  
 the pain, inconvenience, and risks of current invasive  
 techniques.

From the foregoing, it will be appreciated that the  
 30 present invention provides apparatus and methods for  
 noninvasive blood glucose monitoring which avoids the  
 inconvenience and risks associated with traditional  
 invasive blood glucose monitoring techniques.



1        Additionally the present invention provides apparatus  
and methods for noninvasive blood glucose monitoring which  
provide reproducible and accurate correlation with actual  
blood glucose levels.

5        The present invention also provides apparatus and  
methods for noninvasive blood glucose monitoring which  
provide rapid results in sufficient time to administer  
appropriate medication.

         The present invention may be embodied in other  
10 specific forms without departing from its spirit or  
essential characteristics. The described embodiments are  
to be considered in all respects only as illustrative and  
not restrictive. The scope of the invention is, therefore,  
indicated by the appended claims rather than by the  
15 foregoing description. All changes which come within the  
meaning and range of equivalency of the claims are to be  
embraced within their scope.

         What is claimed is:

20

25

30

35

- 1        1.    An apparatus for noninvasive blood glucose  
monitoring comprising:  
         a quantity of glucose receiving medium comprising  
         a permeation enhancer capable of increasing the  
5        glucose permeability across an epithelial membrane;  
         means for supporting the glucose receiving  
         medium; and  
         means for temporarily positioning the glucose  
         receiving medium against the epithelial membrane.
- 10       2.    An apparatus for noninvasive blood glucose  
monitoring as defined in claim 1, wherein the means for  
supporting the glucose receiving medium comprises a housing  
defining a receiving chamber therein and an opening to said  
15       receiving chamber.
3.    An apparatus for noninvasive blood glucose  
monitoring as defined in claim 2, further comprising a  
handle attached to the housing to facilitate positioning of  
20       the apparatus against the mucosal membrane and removal of  
         the apparatus.
4.    An apparatus for noninvasive blood glucose  
monitoring as defined in claim 2, further comprising means  
25       for accessing the receiving chamber such that glucose  
         receiving medium may be introduced into the receiving  
         chamber or removed therefrom while the housing is  
         positioned against the mucosal membrane.
- 30       5.    An apparatus for noninvasive blood glucose  
monitoring as defined in claim 1, wherein the means for  
supporting the glucose receiving medium and the means for  
temporarily positioning the glucose receiving medium  
against the mucosal membrane comprise a hydrogel.

1

6. An apparatus for noninvasive blood glucose monitoring as defined in claim 1, wherein the permeation enhancer comprises a natural bile salt.

5

7. An apparatus for noninvasive blood glucose monitoring as defined in claim 1, wherein the glucose receiving medium comprises water.

10

8. An apparatus for noninvasive blood glucose monitoring as defined in claim 1, wherein the glucose receiving medium comprises a cream.

15

9. An apparatus for noninvasive blood glucose monitoring as defined in claim 1, wherein the glucose receiving medium comprises a suspension.

20

10. An apparatus for noninvasive blood glucose monitoring as defined in claim 1, wherein the glucose receiving medium comprises an emulsion.

25

11. An apparatus for noninvasive blood glucose monitoring as defined in claim 1, wherein the glucose receiving medium comprises a semisolid composition.

30

12. An apparatus for noninvasive blood glucose monitoring as defined in claim 1, wherein the glucose receiving medium comprises a composition capable of reacting with glucose to form a substantially insoluble product.

35

13. An apparatus for noninvasive blood glucose monitoring as defined in claim 1, further comprising means for regulating the glucose permeation rate in order to

1 provide a calibrated permeation rate despite variations in  
glucose permeation from patient to patient and from time to  
time.

5 14. An apparatus for noninvasive blood glucose  
monitoring comprising:

a quantity of glucose receiving medium comprising  
a permeation enhancer capable of increasing the  
glucose permeability across a mucosal membrane;

10 means for supporting the glucose receiving  
medium; and

means for temporarily positioning the glucose  
receiving medium against the mucosal membrane.

15 15. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the means for  
supporting the glucose receiving medium comprises a housing  
defining a receiving chamber therein and an opening to said  
receiving chamber.

20

16. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 15, further comprising a  
handle attached to the housing to facilitate positioning of  
the apparatus against the mucosal membrane and removal of

25 the apparatus.

17. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 15, further comprising means  
for accessing the receiving chamber such that glucose  
30 receiving medium may be introduced into the receiving  
chamber or removed therefrom while the housing is  
positioned against the mucosal membrane.

1        18. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the means for  
supporting the glucose receiving medium and the means for  
temporarily positioning the glucose receiving medium  
5 against the mucosal membrane comprise a hydrogel.

19. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 18, wherein the hydrogel  
comprises hydroxypropylcellulose.  
10

20. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 18, wherein the hydrogel  
comprises carbopol.

15        21. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the permeation  
enhancer comprises a natural bile salt.

22. An apparatus for noninvasive blood glucose  
20 monitoring as defined in claim 14, wherein the permeation  
enhancer comprises sodium cholate.

23. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the permeation  
25 enhancer comprises sodium dodecyl sulfate.

24. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the permeation  
enhancer comprises sodium deoxycholate.  
30

25. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the permeation  
enhancer comprises taurodeoxycholate.

1        26. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the permeation  
enhancer comprises sodium glycocholate.

5        27. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the permeation  
enhancer comprises a fatty acid.

10       28. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the permeation  
enhancer comprises a saturated fatty acid.

15       29. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the permeation  
enhancer comprises an unsaturated fatty acid.

20       30. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the permeation  
enhancer comprises a surfactant.

31. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the permeation  
enhancer comprises an ionic surfactant.

25       32. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the permeation  
enhancer comprises a nonionic surfactant.

30       33. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the permeation  
enhancer comprises a synthetic permeation enhancer.

1        34. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the glucose  
receiving medium comprises water.

5        35. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the glucose  
receiving medium comprises a cream.

10       36. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the glucose  
receiving medium comprises a suspension.

15       37. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the glucose  
receiving medium comprises an emulsion.

20       38. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the glucose  
receiving medium comprises a semisolid composition.

25       39. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, wherein the glucose  
receiving medium comprises a composition capable of  
reacting with glucose to form a substantially insoluble  
product.

30       40. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 14, further comprising means  
for regulating the glucose permeation rate in order to  
provide a calibrated permeation rate despite variations in  
glucose permeation from patient to patient and from time to  
time.

1       41. An apparatus for noninvasive blood glucose  
monitoring comprising:

          a quantity of glucose receiving medium comprising  
a permeation enhancer capable of increasing the  
5       glucose permeability across skin;

          means for supporting the glucose receiving  
medium; and

          means for temporarily positioning the glucose  
receiving medium against the skin.

10

          42. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 41, wherein the means for  
supporting the glucose receiving medium comprises a housing  
defining a receiving chamber therein and an opening to said  
15       receiving chamber.

          43. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 42, further comprising means  
for accessing the receiving chamber such that glucose  
20       receiving medium may be introduced into the receiving  
chamber or removed therefrom while the housing is  
positioned against the skin.

          44. An apparatus for noninvasive blood glucose  
25       monitoring as defined in claim 41, wherein the permeation  
enhancer comprises a natural bile salt.

          45. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 41, wherein the permeation  
30       enhancer comprises DMSO.

          46. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 41, wherein the permeation  
enhancer comprises ethanol.

35



1

47. An apparatus for noninvasive blood glucose monitoring as defined in claim 41, wherein the permeation enhancer comprises a fatty acid.

5

48. An apparatus for noninvasive blood glucose monitoring as defined in claim 41, wherein the permeation enhancer comprises a surfactant.

10

49. An apparatus for noninvasive blood glucose monitoring as defined in claim 41, wherein the permeation enhancer comprises a synthetic permeation enhancer.

50. An apparatus for noninvasive blood glucose monitoring as defined in claim 41, wherein the glucose receiving medium comprises water.

51. An apparatus for noninvasive blood glucose monitoring as defined in claim 41, wherein the glucose receiving medium comprises a cream.

52. An apparatus for noninvasive blood glucose monitoring as defined in claim 41, wherein the glucose receiving medium comprises a suspension.

25

53. An apparatus for noninvasive blood glucose monitoring as defined in claim 41, wherein the glucose receiving medium comprises an emulsion.

30

54. An apparatus for noninvasive blood glucose monitoring as defined in claim 41, wherein the glucose receiving medium comprises a semisolid composition.

35

1        55. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 41, wherein the glucose  
receiving medium comprises a composition capable of  
reacting with glucose to form a substantially insoluble  
5 product.

      56. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 41, further comprising means  
for regulating the glucose permeation rate in order to  
10 provide a calibrated permeation rate despite variations in  
glucose permeation from patient to patient and from time to  
time.

      57. An apparatus for noninvasive blood glucose  
15 monitoring comprising:

      a housing defining a receiving chamber therein  
and an opening to said receiving chamber;

      a quantity of glucose receiving medium located  
within the receiving chamber, said glucose receiving  
20 medium comprising a permeation enhancer capable of  
increasing the glucose permeability across an  
epithelial membrane; and

      means for temporarily positioning the housing  
against the epithelial membrane, such that the opening  
25 to the receiving chamber is positioned over the  
epithelial membrane.

      58. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 57, wherein the glucose  
30 receiving medium comprises water.

      59. An apparatus for noninvasive blood glucose  
monitoring as defined in claim 57, wherein the glucose  
receiving medium comprises a hydrogel.  
35

1

60. An apparatus for noninvasive blood glucose monitoring as defined in claim 57, wherein the glucose receiving medium comprises a semisolid composition.

5

61. An apparatus for noninvasive blood glucose monitoring as defined in claim 57, wherein the glucose receiving medium comprises a composition capable of reacting with glucose to form a substantially insoluble  
10 product.

62. An apparatus for noninvasive blood glucose monitoring as defined in claim 57, further comprising means for regulating the glucose permeation rate in order to  
15 provide a calibrated permeation rate despite variations in glucose permeation from patient to patient and from time to time.

63. An apparatus for noninvasive blood glucose  
20 monitoring as defined in claim 57, wherein the permeation enhancer comprises a natural bile salt.

64. An apparatus for noninvasive blood glucose monitoring as defined in claim 57, wherein the permeation  
25 enhancer comprises a synthetic permeation enhancer.

65. An apparatus for noninvasive blood glucose monitoring as defined in claim 57, further comprising means for accessing the receiving chamber such that glucose  
30 receiving medium may be introduced into the receiving chamber or removed therefrom while the housing is positioned against the epithelial membrane.

1        66. An apparatus for noninvasive blood glucose monitoring as defined in claim 57, wherein the epithelial membrane comprises a mucosal membrane.

5        67. An apparatus for noninvasive blood glucose monitoring as defined in claim 57, wherein the epithelial membrane comprises skin.

68. A method for noninvasive blood glucose monitoring  
10 comprising the steps of:

(a) obtaining a quantity of glucose receiving medium comprising a permeation enhancer capable of increasing the glucose permeability across an epithelial membrane, said glucose receiving medium  
15 being supported by a support material;

(b) temporarily positioning the glucose receiving medium against the epithelial membrane; and

(c) allowing sufficient time to pass such that blood glucose from the interstitial fluid in  
20 equilibrium with the capillary blood vessels perfusing the epithelial tissue diffuses across the epithelial membrane into the receiving medium.

69. A method for noninvasive blood glucose monitoring  
25 as defined in claim 68, further comprising the step of testing the glucose receiving medium for the presence of glucose.

70. A method for noninvasive blood glucose monitoring  
30 as defined in claim 68, wherein the glucose receiving medium is supported by a hydrogel.

1        71. A method for noninvasive blood glucose monitoring  
as defined in claim 68, wherein the permeation enhancer  
comprises a natural bile salt.

5        72. A method for noninvasive blood glucose monitoring  
as defined in claim 68, wherein the permeation enhancer  
comprises a synthetic permeation enhancer.

73. A method for noninvasive blood glucose monitoring  
10 comprising the steps of:

(a) obtaining a quantity of glucose receiving  
medium comprising a permeation enhancer capable of  
increasing the glucose permeability across a mucosal  
membrane, said glucose receiving medium being  
15 supported by a support material;

(b) temporarily positioning the glucose  
receiving medium against the mucosal membrane;

(c) allowing sufficient time to pass such that  
blood glucose from the interstitial fluid in  
20 equilibrium with the capillary blood vessels perfusing  
the mucosal tissue diffuses across the mucosal  
membrane into the receiving medium; and

(d) testing the glucose receiving medium for the  
presence of glucose.  
25

74. A method for noninvasive blood glucose monitoring  
as defined in claim 73, wherein the glucose receiving  
medium is supported by a hydrogel.

30        75. A method for noninvasive blood glucose monitoring  
comprising the steps of:

(a) obtaining a quantity of glucose receiving  
medium comprising a permeation enhancer capable of  
increasing the glucose permeability across skin, said  
35

1 glucose receiving medium being supported by a support  
material and said glucose receiving medium having a  
substantially negligible glucose concentration;

5 (b) temporarily positioning the glucose  
receiving medium against the skin;

(c) allowing sufficient time to pass such that  
blood glucose from the interstitial fluid in  
equilibrium with the capillary blood vessels perfusing  
the skin diffuses across the mucosal membrane into the  
10 receiving medium; and

(d) testing the glucose receiving medium for the  
presence of glucose.

76. A method for noninvasive blood glucose monitoring  
15 as defined in claim 75, wherein the permeation enhancer  
comprises DMSO.

77. A method for noninvasive blood glucose monitoring  
as defined in claim 75, wherein the permeation enhancer  
20 comprises ethanol.

25

30

35

1/19

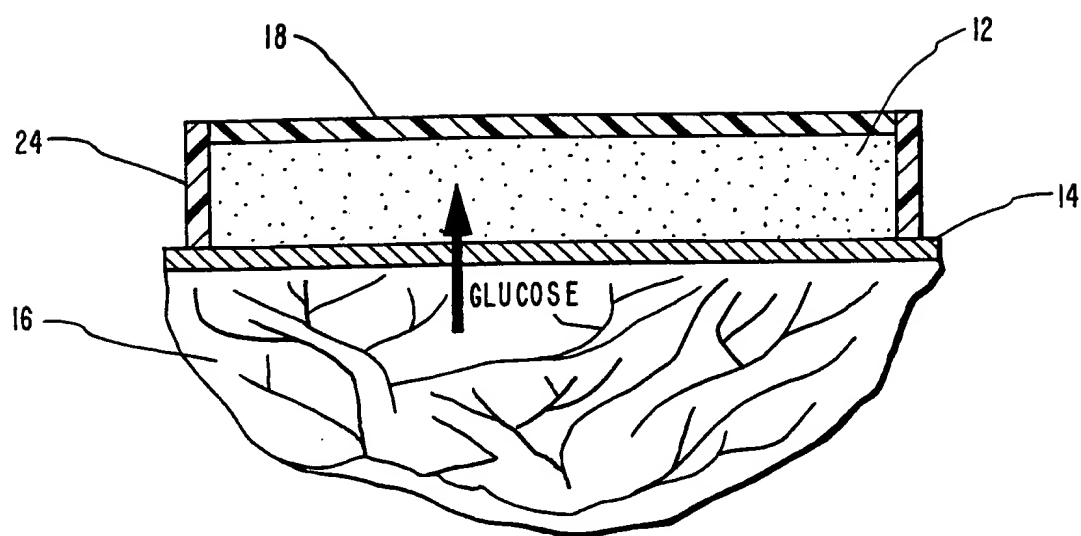


FIG. 1

2/19

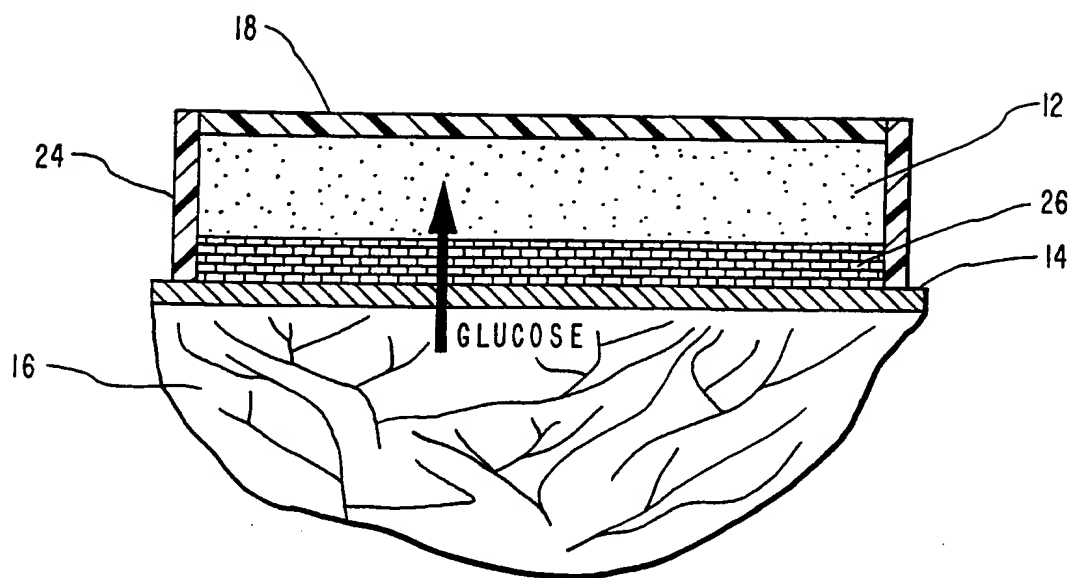


FIG. 2



3/19

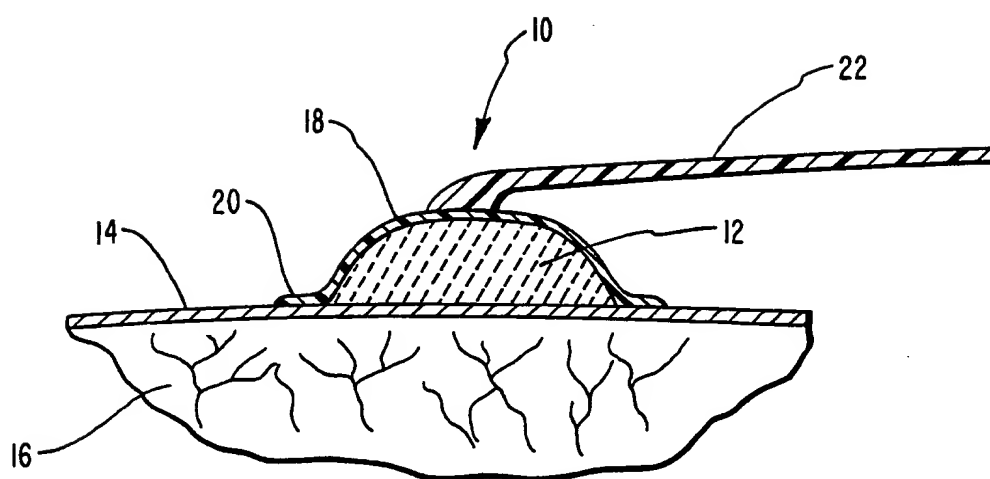


FIG. 3

4/19

2% SODIUM CHOLATE, RECEIVING MEDIUM: WATER

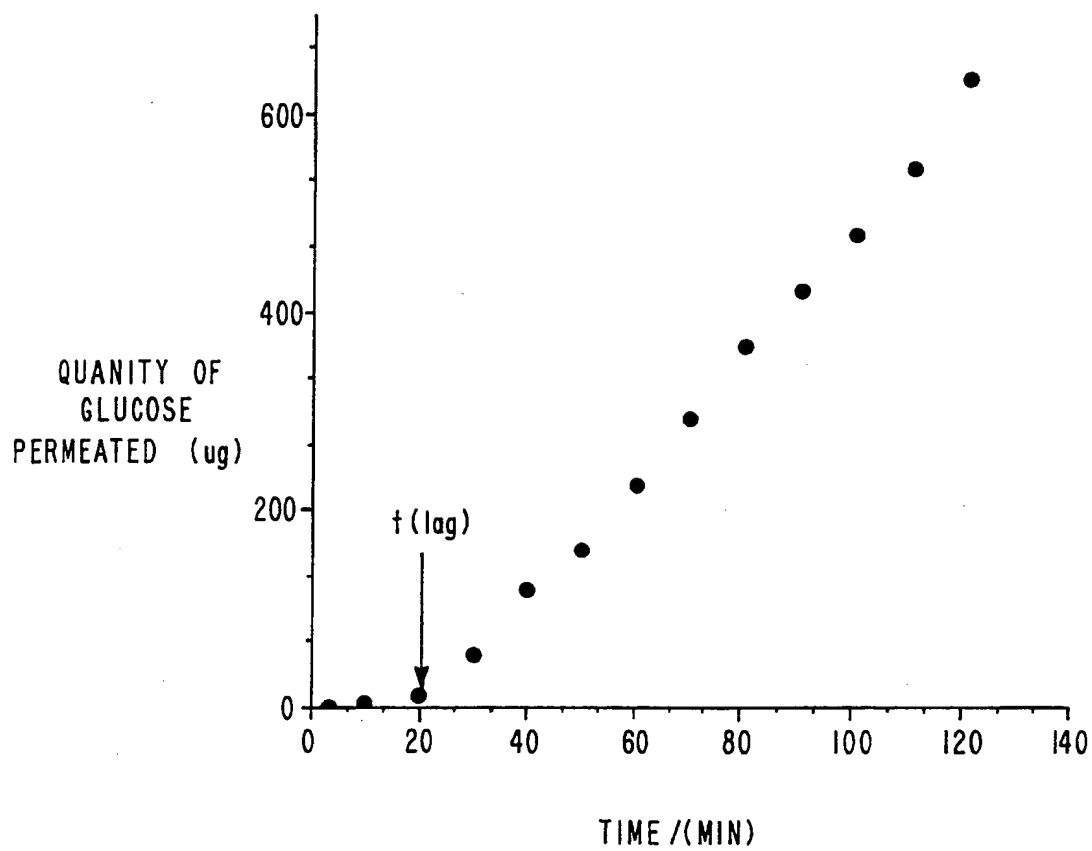


FIG. 4

5/19

2% SODIUM CHOLATE, RECEIVING MEDIUM: WATER

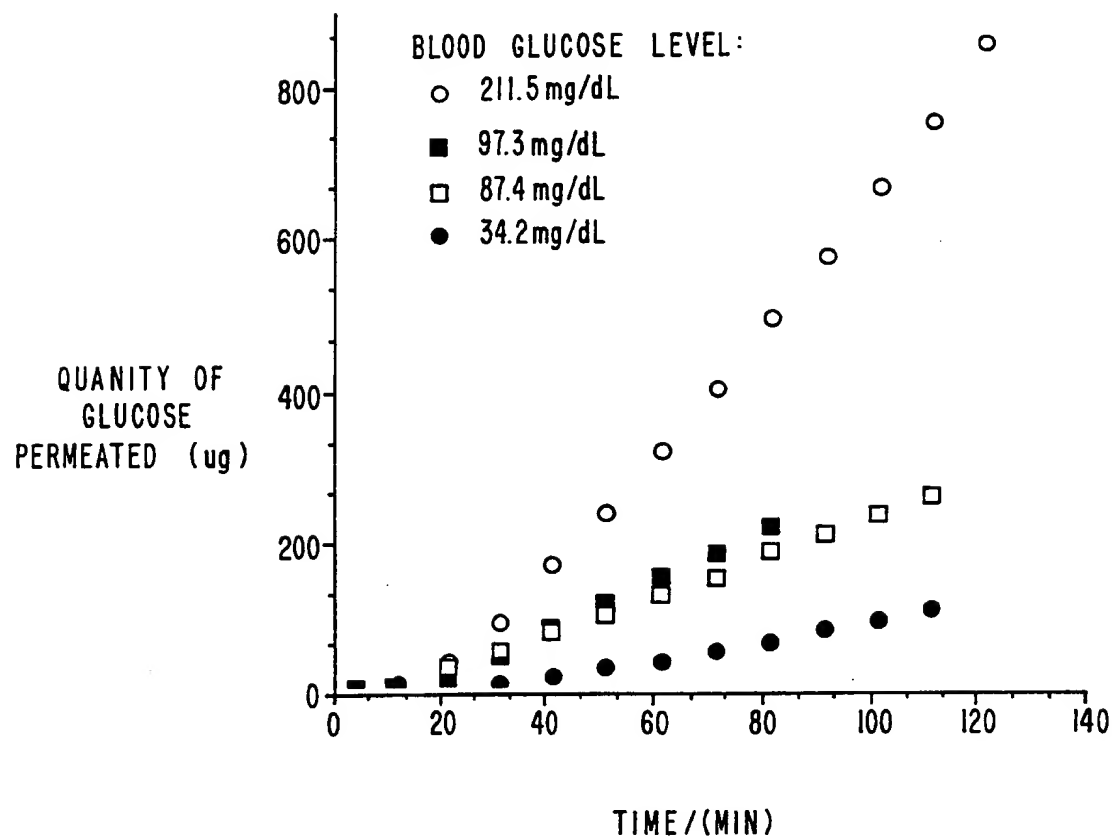


FIG. 5

6/19

2% SODIUM CHOLATE, RECEIVING MEDIUM: WATER

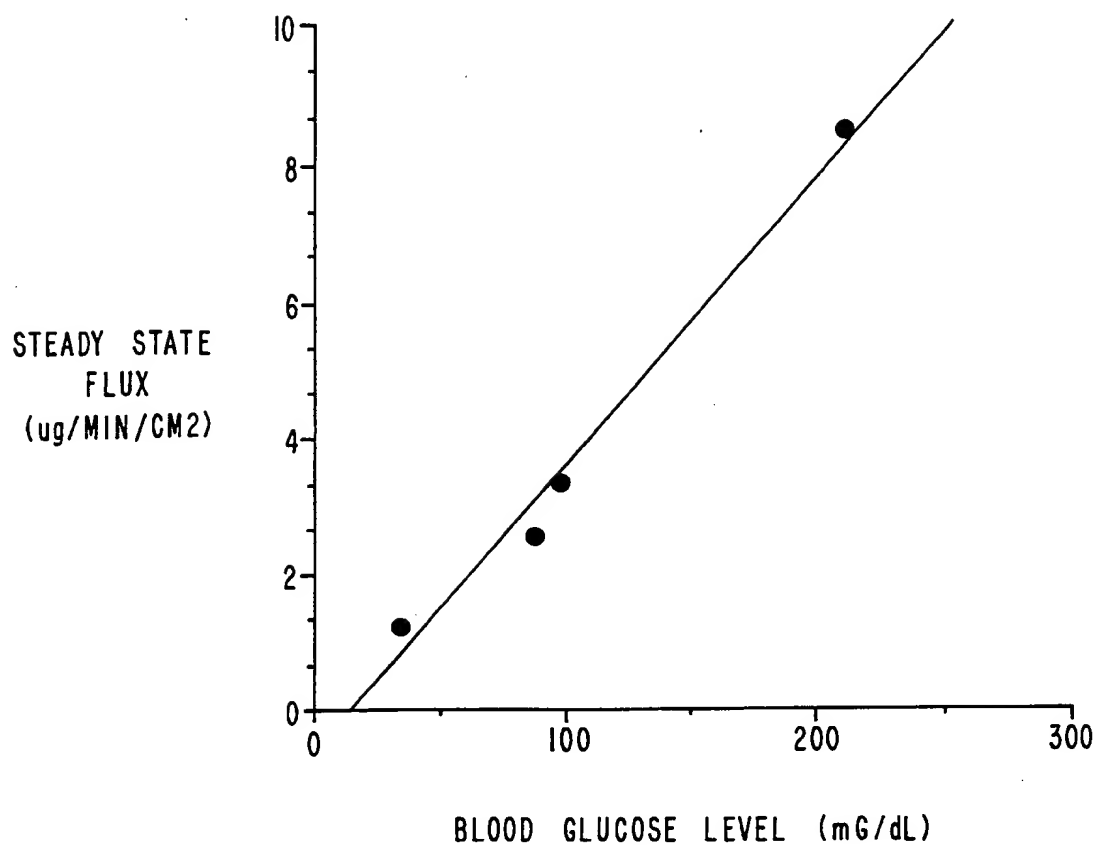


FIG. 6

7/19

## EFFECT OF PERMEATION ENHANCER

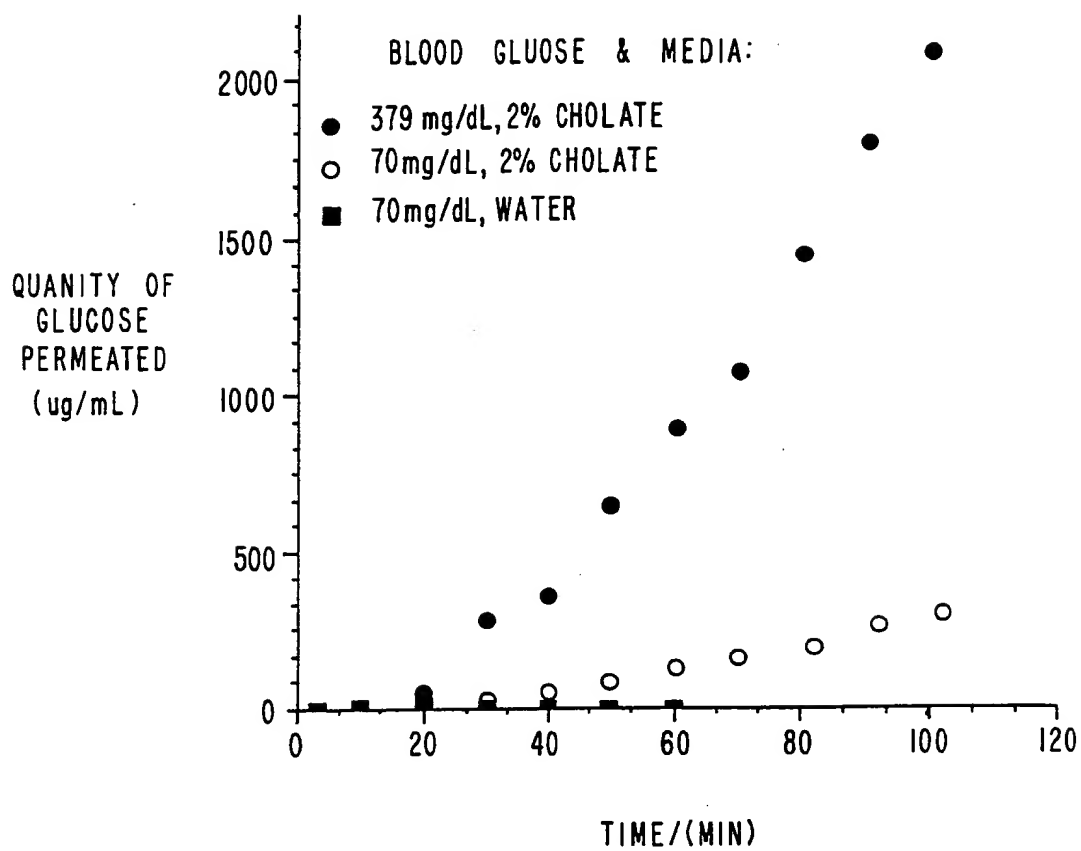


FIG. 7

8/19

2% SODIUM CHOLATE IN WATER &amp; 30% ETHANOL

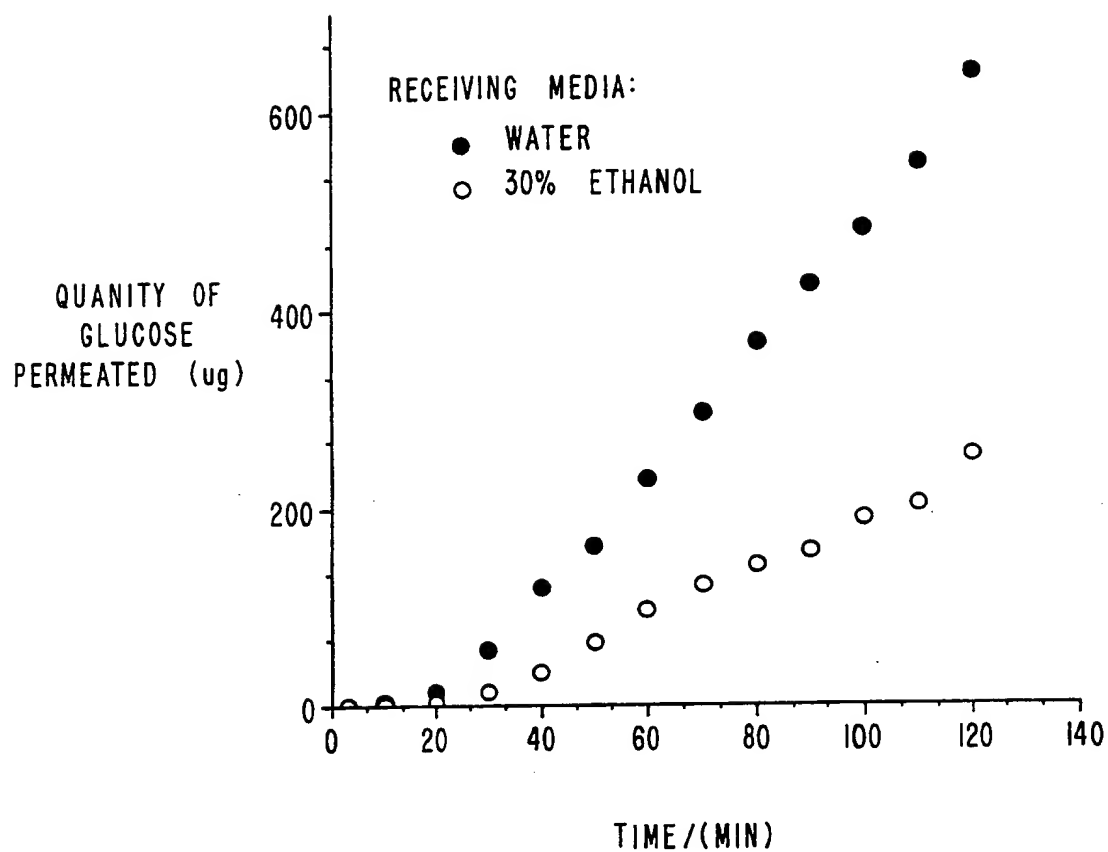


FIG. 8

9/19

2% SODIUM CHOLATE, RECEIVING MEDIUM: WATER

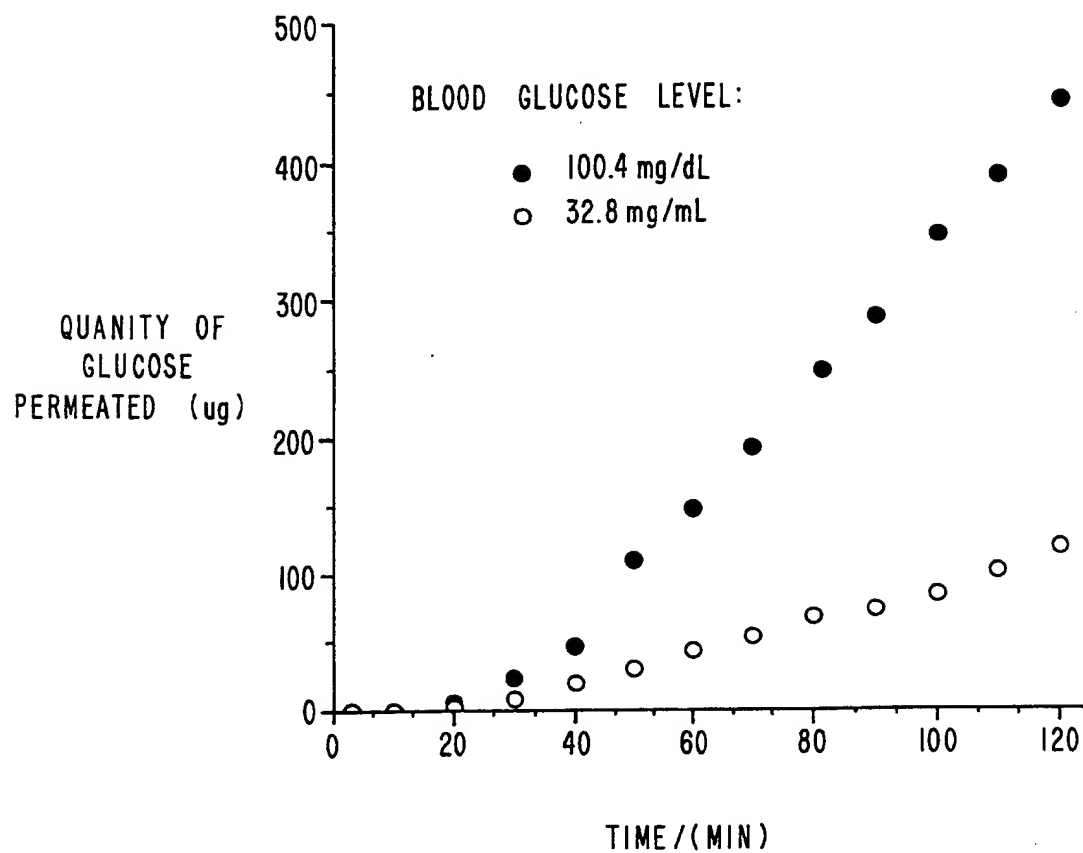


FIG. 9

10/19

8% SODIUM CHOLATE, RECEIVING MEDIUM: HYDROGEL

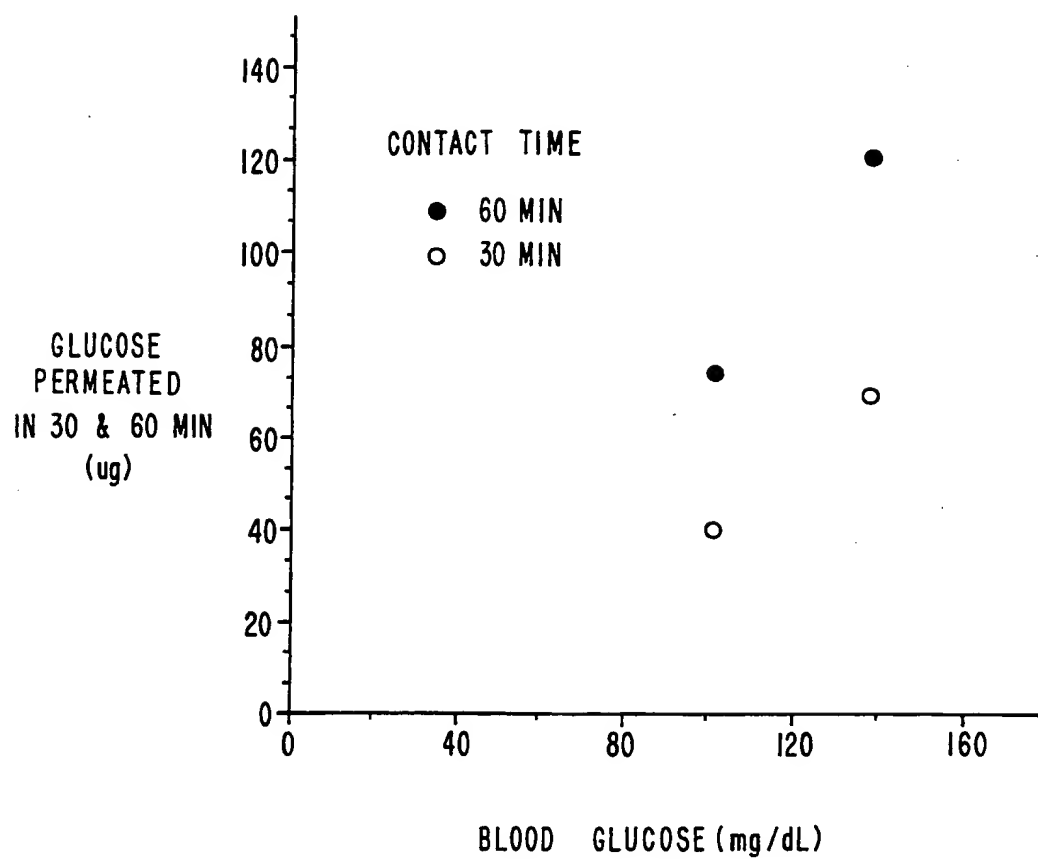


FIG. 10



11/19

4% SODIUM CHOLATE, RECEIVING MEDIUM: HYDROGEL

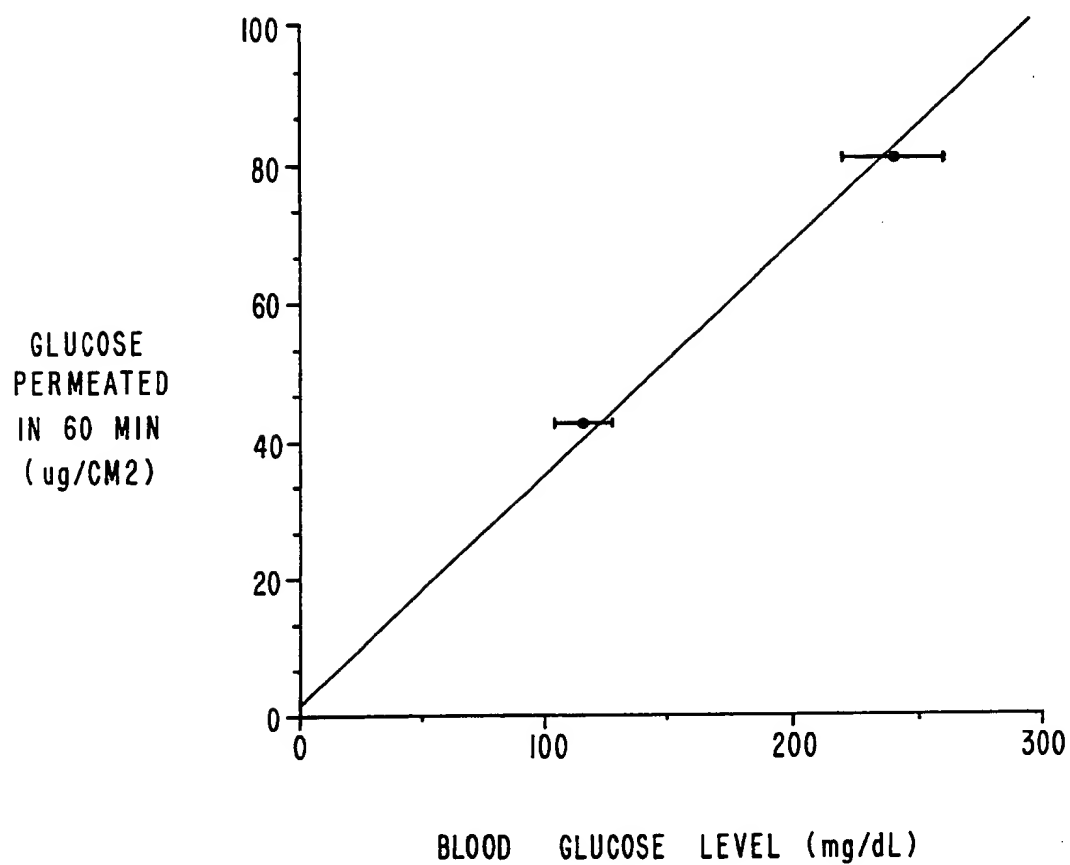


FIG. 11

12/19

8% SODIUM CHOLATE, RECEIVING MEDIUM: HYDROGEL

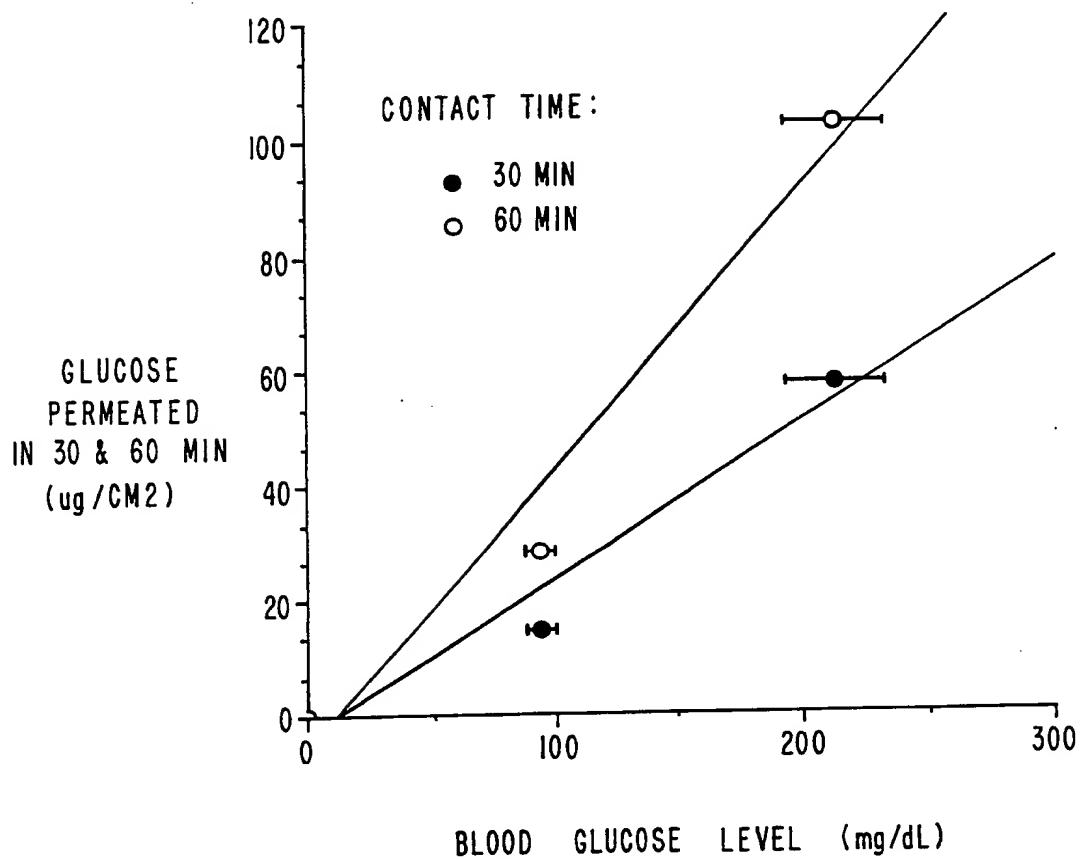


FIG. 12

13/19

8% SODIUM CHOLATE, RECEIVING MEDIUM: HYDROGEL

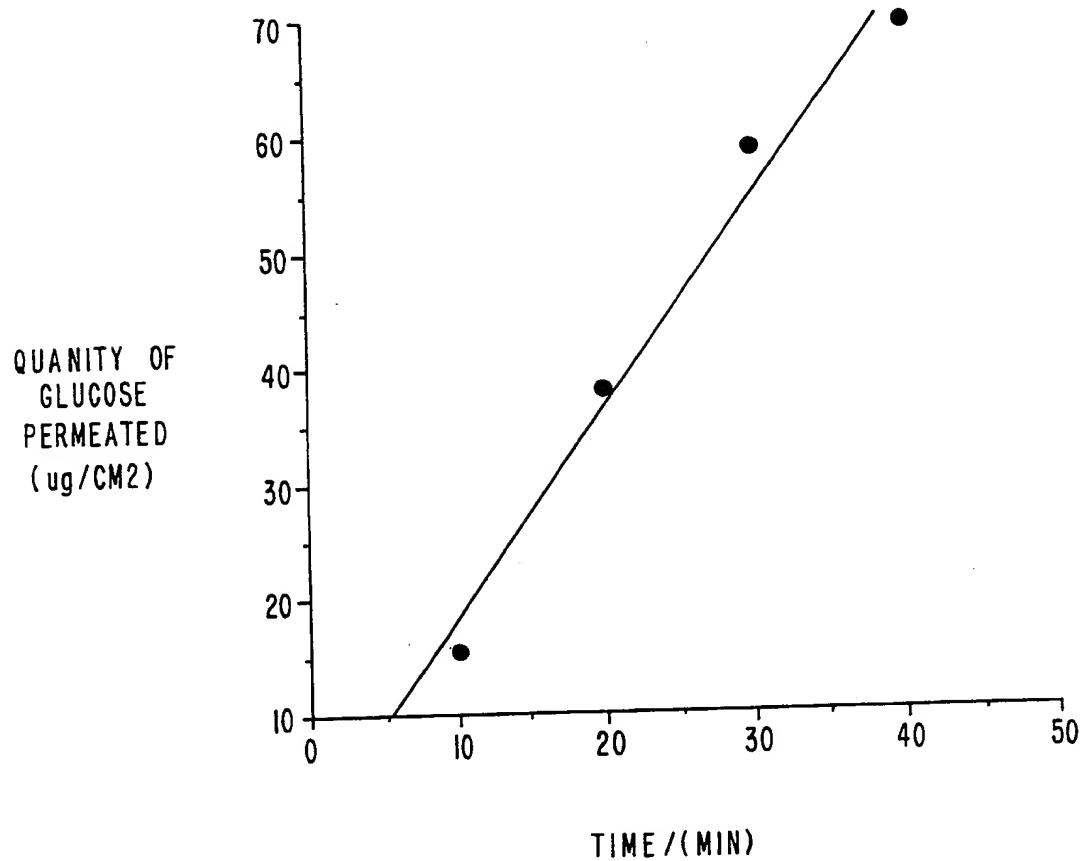


FIG. 13

14/19

16% SODIUM CHOLATE, RECEIVING MEDIUM: HYROGEL

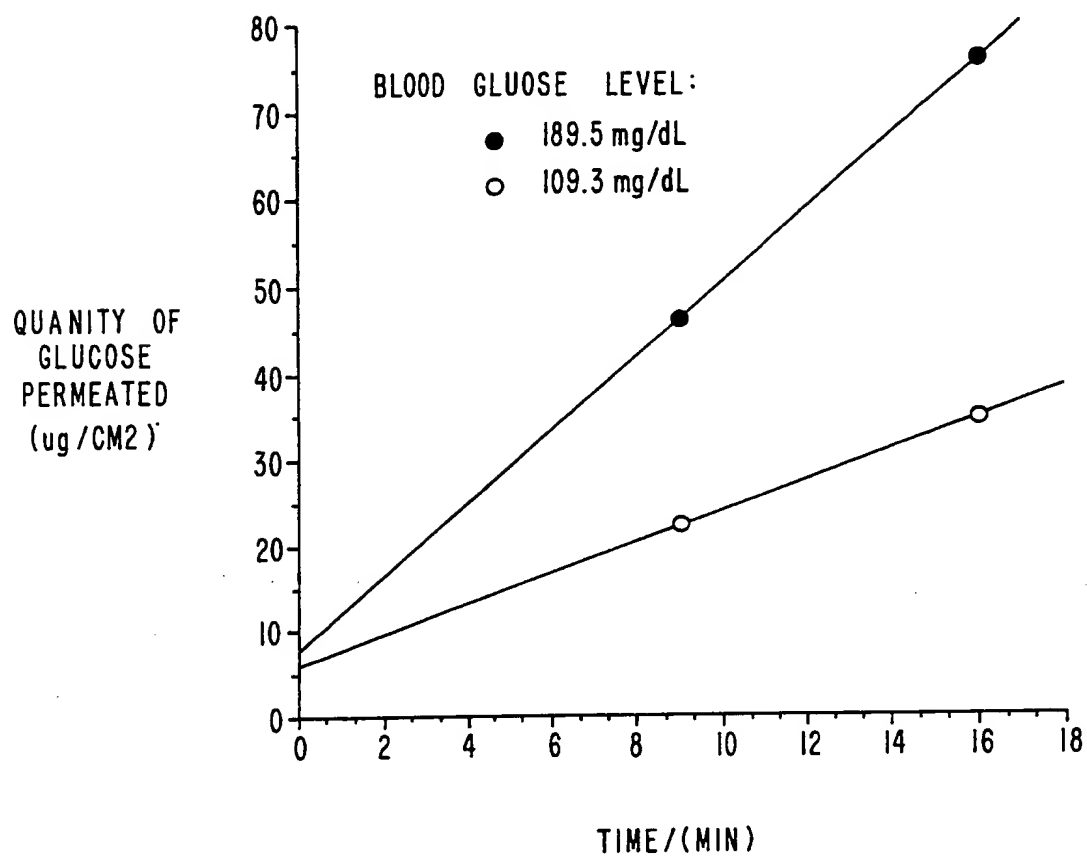


FIG. 14

15/19

## WATER &amp; HYDROGEL AS GLUCOSE RECEIVING MEDIA

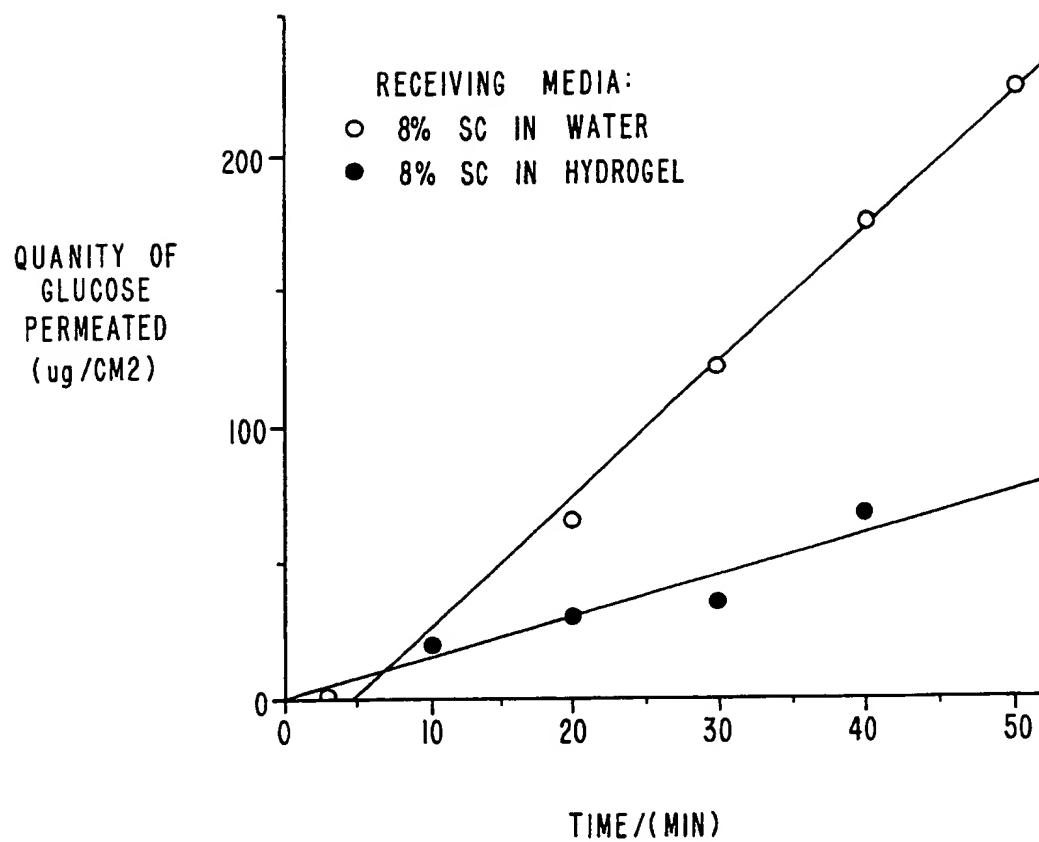


FIG. 15

16/19

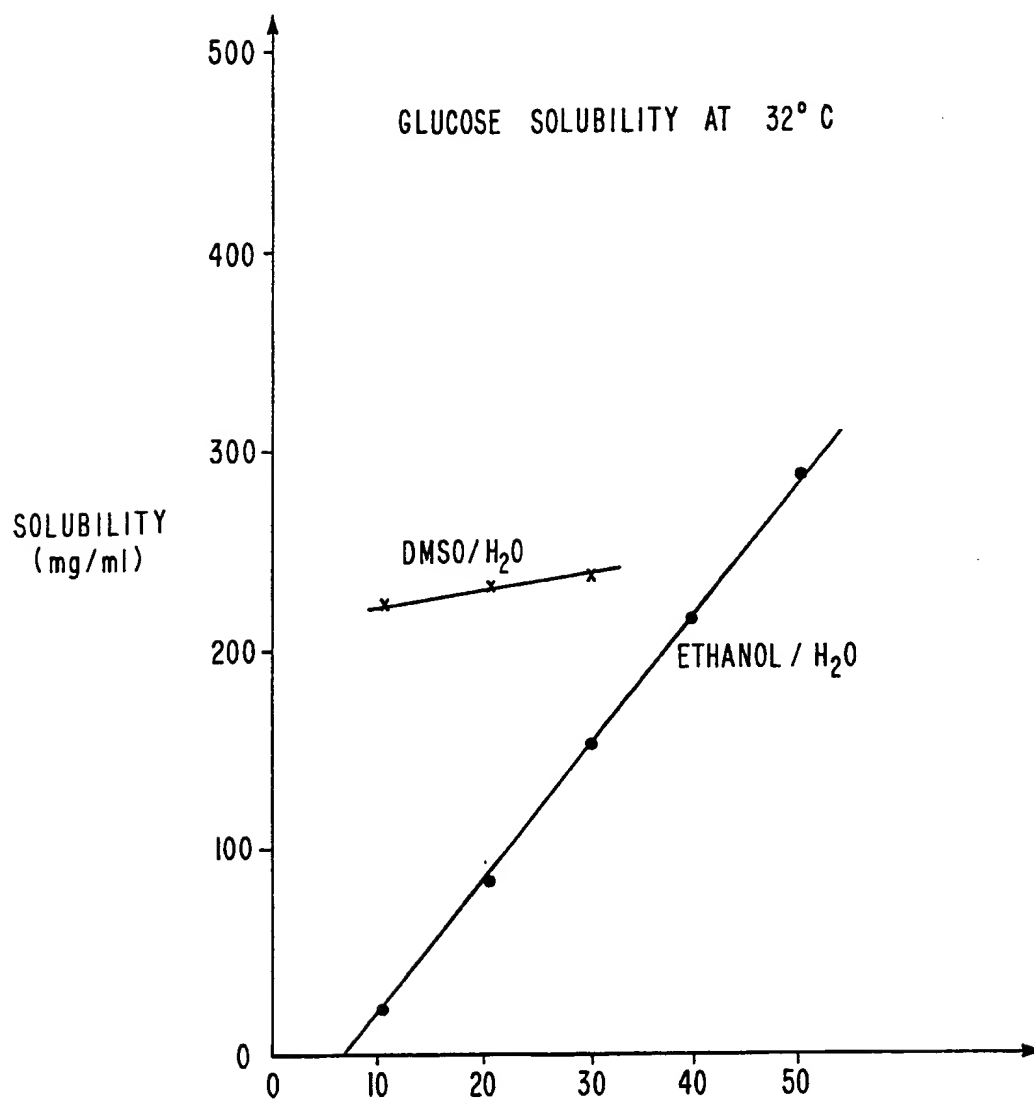
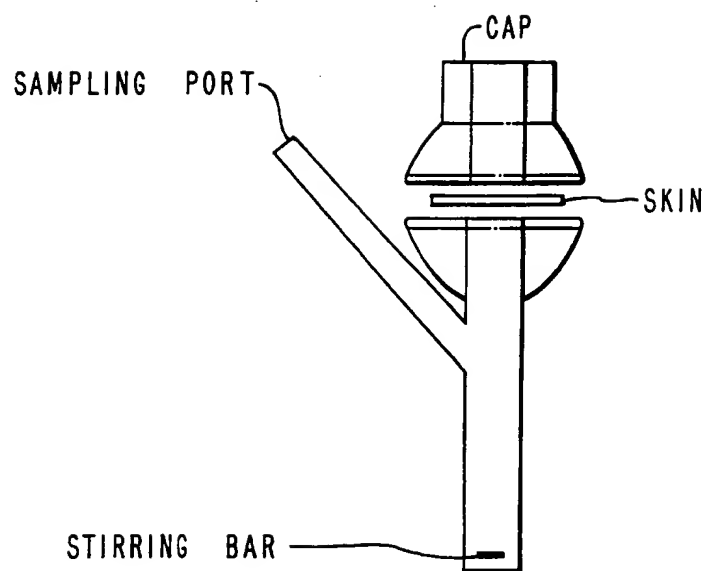
PERCENT WATER IN ETHANOL / WATER MIXTURE  
AND DMSO / WATER MIXTUREGLUCOSE SOLUBILITY AS A FUNCTION OF  
WATER CONTENT IN THE EtOH / H<sub>2</sub>O AND  
DMSO / H<sub>2</sub>O SYSTEM AT 32° C

FIG. 16

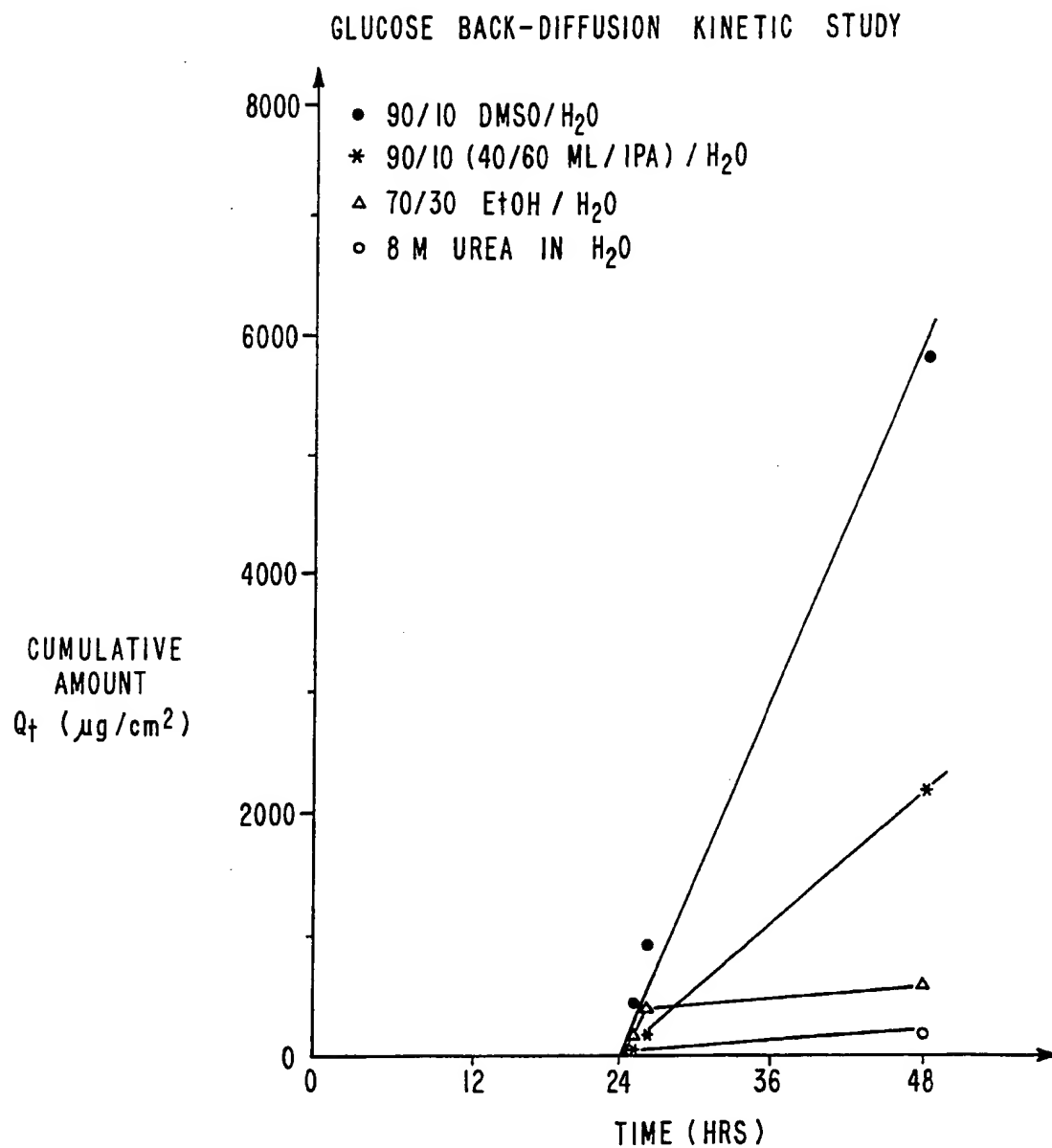
17/19



NON-JACKETED GLASS DIFFUSION CELL

FIG. 17

18/19



GLUCOSE BACK-DIFFUSION KINETICS IN THE  
ENHANCER PRE-TREATED STUDIES

FIG. 18



19/19

## GLUCOSE BACK-DIFFUSION KINETIC STUDY

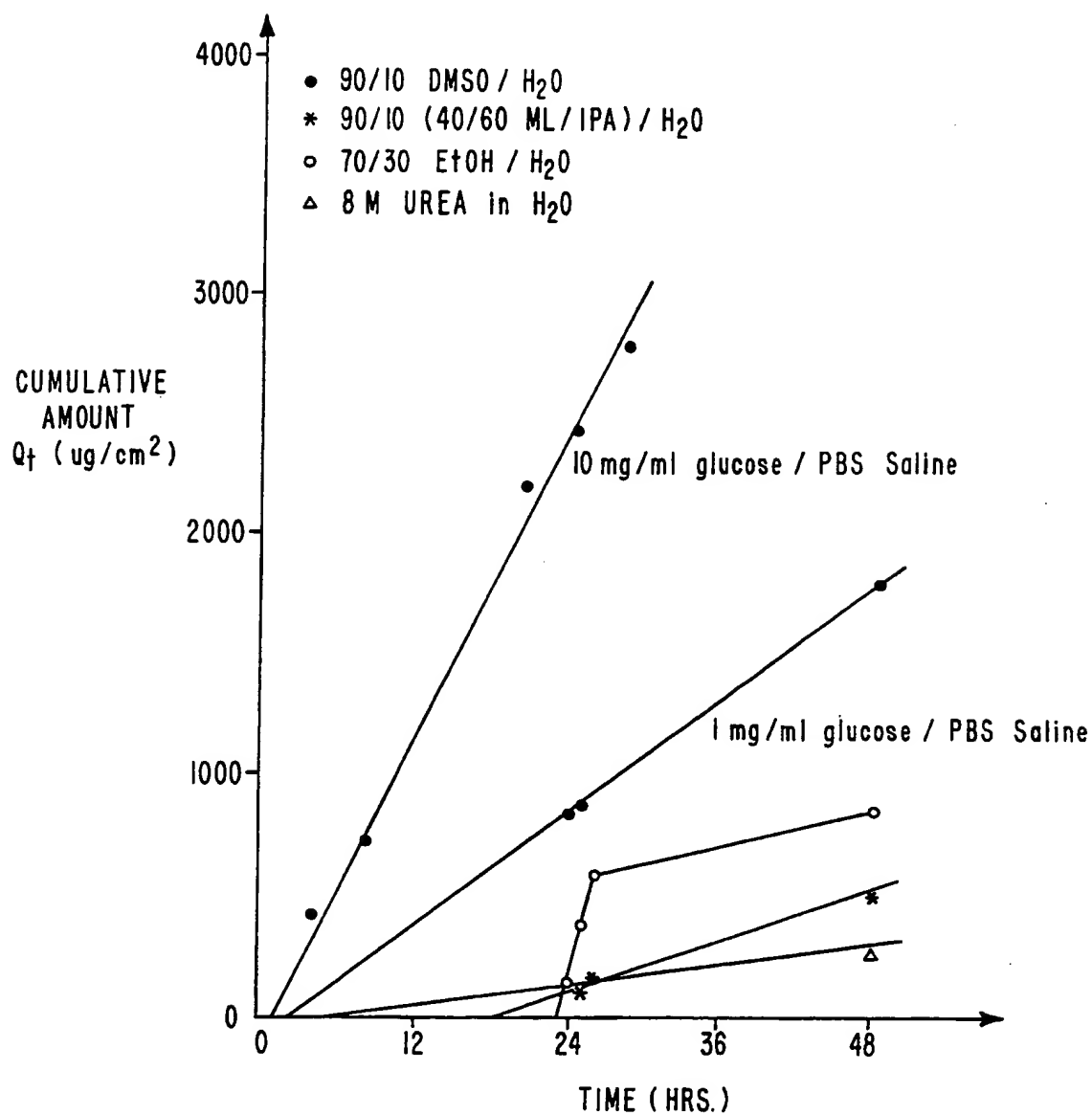
GLUCOSE BACK-DIFFUSION KINETICS EMPLOYING  
TTI ENHANCER SYSTEMS

FIG. 19